FOREST Science

A QUARTERLY JOURNAL OF RESEARCH AND TECHNICAL PROGRESS

Volume 5 - Number 2 - June 1959

PUBLISHED BY SOCIETY OF AMERICAN FORESTE

WASHINGTON, D. C.

FOREST SCIENCE is published quarterly by the Society of American Foresters with the cooperation of forestry research and educational organizations.

The journal assumes no responsibility for the statements and opinions expressed by contributors. The editors cannot be responsible for loss or damage of manuscripts and accompanying matter.

Correspondence concerning manuscripts submitted for publication and other editorial matters should be addressed to Prof. Stephen H. Spurr, School of Natural Resources, University of Michigan, Ann Arbor, Michigan.

Other correspondence, including subscriptions, remittances, reprint orders, and inquiries concerning the status of manuscripts accepted for publication, should be addressed to the Society of American Foresters, Mills Building, Washington 6, D. C.

Subscription price is $6.00 per year for complete volumes (one per calendar year), and $2.00 for single number. Add $0.25 for postage to Canadian addresses and $0.50 for postage to other foreign addresses.

The pages of FOREST SCIENCE are open to all qualified contributors. Preference will be given to papers devoted to the presentation and discussion of the results of original research. Authors are expected to bear the cost of that portion of their papers in excess of 16 printed pages in length and of tabular and illustrative material constituting more than 20 percent of the length of the paper.

Papers of 32 printed pages or longer (approximately 20,000 words or about 65 pages of manuscript) will be considered for publication as *Forest Science Monographs* under the same editorial procedures followed for shorter contributions. The monographs will be printed with covers, distributed without charge to the subscribers as supplements to regular issues, listed in the table of contents of the appropriate issue of FOREST SCIENCE, and included in the annual index. The entire cost of printing and mailing each monograph (but not editorial costs) must be met by the author or other agency.

Board of Editor

Stephen H. Spu
Edit
School of Natural Resour
University of Michig

Henry Clepp
F. H. Ey
Society of American Forest

V. L. Harp
Forest Service, U. S. Department of Agricult

Business Manag
Miss L. Audrey Warn
Society of American Forest

Copyright, 1959, by the Society of American Foreste

Second class postage paid at Washington, D. C., and at additional mailing offi

Printed in the United States of Amer

FOREST *Science*

A Quarterly Journal of Research and Technical Progress

The Columbian Timber Beetle and Associated Fungi in White Oak
Charles L. Wilson 114

Absorption Rates of 2,4,5-T Tagged with Carbon-14
Laurence C. Walker, Edwin Beck, Erwin Dumbroff 128

Photoperiodic Control of Bud Development in Coulter Pine and Big-cone Douglas-Fir
Henry Hellmers 138

Influence of Potassium Fertilizer Level on Red Pine Planted at Various Spacings on a Potassium-Deficient Site
S. O. Heiberg, L. Leyton, H. Loewenstein 142

A System for Analysis of Forest Succession
John P. Decker 154

In Vitro Culture of Pine Roots and the Use of *Pinus serotina* Roots in Metabolic Studies
Robert L. Barnes, Aubrey W. Naylor 158

The Leuco-anthocyanin Distribution in the Genus Pinus
Stanley L. Krugman 169

Germination of Douglas-Fir Seed as Affected by Light, Temperature, and Gibberellic Acid
S. D. Richardson 174

Perennial Infection of Ponderosa Pine by *Elytroderma deformans*
Lewis F. Roth 182

The Biology and Ecology of the Balsam Gall Midge in Wisconsin
Ronald L. Giese, Daniel M. Benjamin 193

REVIEWS: Tropical silviculture, 137; The native pinewoods of Scotland, 157; Data for planning woodland opportunities on West Tennessee farms, 191.

FOREST SCIENCE MONOGRAPH 1: Private Forestry in Norway—A Case Study in Small Woodland Management by John A. Zivnuska. (Mailed with this issue of *Forest Science* as a separate.)

Advisory Board

Harry A. Fowells
Scott S. Pauley
Carl H. Stoltenberg
John A. Zivnuska

F. Paul Keen
François Mergen
J. Harry G. Smith
Donald P. White

George H. Hepting
T. T. Kozlowski
Leslie W. Orr
Robert Zahner

Volume 5
June, 1959
Number 2

The Columbian Timber Beetle And Associated Fungi in White Oak

BY
CHARLES L. WILSON

THE COLUMBIAN TIMBER BEETLE (*Corthylus columbianus* Hopk.) is an important pest in West Virginia. It is considered by many processors of white oak (*Quercus alba* L.) and especially by manufacturers of veneer and cooperage as their most destructive pest, causing defects that are commonly called "flag - worms," "grease - spots," "patch-worms," and "black-holes." Except for the original descriptions of this scolytid beetle and its damage by A. D. Hopkins (1893, 1894a), little information is available concerning it and no information has been published on its fungus associates. Although *C. columbianus* is reported to attack several tree species, white oak was the only host considered in this study.

This beetle and its damage are unique in several respects. Unlike most scolytid beetles, *C. columbianus* attacks vigorous trees. After the beetles have raised their young within the sapwood of a living tree and the new generation has emerged, the subsequent growth of the cambium seals in the galleries. Each year more wood is laid down over the gallery until eventually it is deeply imbedded within the heartwood. This allows one to establish with some accuracy the date of entrance of a particular generation of the beetle by counting the annual rings formed after the beetle entered.

The stain associated with most ambrosia beetle galleries extends only a short distance from the gallery and results in little damage to the wood. Such is not the case with the galleries of *C. columbianus*, where the stains are more extensive and there is some modification in the structure and strength of the wood.

C. columbianus is endemic in the United States. Hopkins found damage by the beetle dating back to 1479, "13 years before Columbus discovered America." Although the damage caused by most of our native pests is commonly much less than that caused by introduced species, the Columbian timber beetle causes considerable economic loss. In attacking vigorous trees it apparently affects the continued growth of the tree little, if any, so that at maturity a

---

The author is Assistant Professor of Plant Pathology, Plant Pathology Dept., University of Arkansas, Fayetteville. This account is based in part upon his Ph.D. dissertation and is published with the approval of the Director of the West Virginia Agric. Expt. Station as Scientific Paper No. 583.

The author is indebted to J. G. Leach, R. P. True, C. K. Dorsey, H. L. Barnett and H. L. Hansen for help and encouragement, and to the Division of Forestry of the University of West Virginia who, in cooperation with the State Conservation Commission, made available the trees on Cooper's Rock State Forest. Timber processors in West Virginia and other states were most helpful in supplying information and providing material for study. Thanks are also due to John Hunt, Pacific Northwest For. and Range Expt. Sta., for identifying *C. piceae*, and to W. H. Anderson, U. S. Dept. of Agric. Research Service, for confirming the identification of *C. columbianus*.

tree that appears normal externally may contain 200-300 years of accumulated damage caused by this insect.

Hopkins' reports on the Columbian timber beetle are distributed among several publications one of which (1984a) contains the most thorough treatment. In 1903 he commented interestingly that: "Within recent years, examples of the species which do this work have been exceedingly scarce; consequently but little evidence of its work can be found in the sapwood and outer heartwood of living trees. Therefore, there is no remedy for the old work, and probably no need of trying to combat an insect which is apparently becoming extinct."

Hubbard (1897) included *C. columbianus* among the species of ambrosia beetles of the United States. Although he discussed the ambrosia fungus associated with *C. punctatissimus* Zimm., he did not examine the ambrosia of *C. columbianus*. He made mention only of the dark stain accompanying the galleries.

Since 1909 there have been occasional reports of the occurrence of *C. Columbianus* in various areas (Anon. 1956, 1957), but no critical studies have been made. Lockard *et al.* (1950), in discussing log defects in southern hardwoods, state that spot or flag-worm holes are clean-cut holes about $\frac{1}{32}$- to $\frac{1}{16}$-inch in diameter, typically occurring in pairs. They found these holes to be surrounded by an oval or elongated stained or (in oaks and elms) bleached area or "flag." They stated that white oak over most of its range is free of this defect, but indicated that damage is likely to be found on sites where the soils are dry or lacking in lime.

Materials and Methods

The white oak logs, trees, and stumps studied were primarily from 3 locations, representing northern, southern, and northwestern West Virginia. These locations were Cooper's Rock State Forest near Morgantown, and sawmills near Mount Hope, Cairo, and West Union. All morphological and anatomical studies of *C. columbianus* were made from specimens obtained from a single tree near Morgantown. All fungal isolations made from current galleries and associated stains also came from this same tree.

Malt extract-yeast extract agar medium (10 grams malt extract and 2 grams yeast extract/liter) was used in all isolations. Isolations from the galleries and stained areas were made by flaming the areas to be cultured, cutting small chips of wood with a sterile knife, and transferring them to the medium. *Ceratocystis piceae* (Münch) Bakshi was more easily obtained in pure culture from current galleries by placing pieces of wood containing the galleries in a moist chamber. Under these conditions the *Graphium* stage developed extensively on the wood adjacent to the galleries. Isolations could then be made by touching a piece of agar to the *Graphium* heads and placing the seeded agar on the medium. It was difficult to isolate this fungus in pure culture from chips including portions of the gallery wall because of the rapid growth of other organisms present. Isolations from the beetles were made by allowing them to walk on agar plates; also by crushing the beetles and streaking the fragments on plates. The latter method proved better for the isolation of *Pichia* sp.

Histological studies were made of the intestinal tract of *C. columbianus* and of the wood adjacent to the galleries. Whole beetles, fixed in Zenker's-formol fixative for 24 hours, were washed in running water for 12 hours. A few drops of iodine were added daily to the wash water containing the beetles until the iodine color persisted. The intestinal tract was dissected from these beetles, imbedded in paraffin, sectioned, and mounted on microscope slides using standard procedures. Cut sections ranged from 4 to 12μ in thickness. Stains used included Gram-Glynn bacterial stain, Goodpasture's carbol fuschsin and methylene blue, and the safranin and fast-green combinations. Gram-Glynn's stain proved

the best for defining bacteria and spores within the intestinal tract. Wood to be sectioned was first fixed in FAA for 24 hours or longer and sections 6 to 30μ thick were cut on a sliding microtome.

Results

Nature of the damage by Corthylus columbianus *in white oak.* It is extremely difficult to determine whether a growing white oak tree has been or is currently infested by *C. columbianus.* A tree within Cooper's Rock State Forest that contained numerous current galleries of this beetle presented no apparent signs of infestation until it was felled and cross sections of the bole were examined (Fig. 1). Since beetles enter through crevices in the bark their entrance holes are difficult to see even upon close inspection of felled trees. "Wet stains," usually small and not visible from a distance and similar to those described by Hopkins (1894a), were observed in areas of the bark where the beetles entered (Fig. 8). Frequently the beetles excavate a small cavity in the bark, near the point at which the entrance hole will be made. However, this excavated area usually cannot be seen until some of the top layers of bark are removed. When the outer bark is cut away from a white oak currently infested, the most recent entrance holes become quite visible in the inner bark and adjacent wood (Figs. 5, 6, 7). A slime-flux often exudes from galleries currently occupied by *C. columbianus* (Fig. 5). After the galleries have been vacated, callus tissue forms around the entrance holes (Fig. 6). The following year these openings appear as longitudinal slits at the cambial surface (Fig. 7). These slits may be slightly open and bleeding. In later years entrance holes become difficult or impossible to distinguish even in debarked logs.

The most obvious signs of the presence of *C. columbianus* in a log or felled tree are the characteristic "flags" that are seen in cross sections of the bole (Figs. 1, 2, 10). These "flags," resulting from stains that extend chiefly upward and downward from the beetle galleries, conform to the shape of the galleries and mark their locations. Light brown when first exposed to the air, the stains in the sapwood later become purplish black (Figs. 1, 10). Stains in the heartwood (Fig. 2) remain brown, but darken slightly. They may extend as far as 12 inches above and below the galleries. Occasionally the stains from 2 or more galleries meet and coalesce (Fig. 3). In one case noted the stains from 4 superimposed galleries coalesced vertically, forming a continuous 34-inch column of stain in the wood. The stain narrows as it extends above and below the galleries, and at its extremities may be limited to only a few vessels. In cross-section the extreme end of the stain from a gallery may appear quite similar to a "mineral stain" or "bird peck" defect.

Although the stains generally extend an equal distance above and below the galleries (Figs. 4, 11), in individual cases they may reach further in one direction than the other. They usually extend 4 to 6 inches from the gallery and apparently reach their maximum length the same year the galleries are initiated. The average length of stains from current, fully-developed galleries was the same as that of galleries found in the heartwood. There is little radial or tangential spread of the stain. Therefore, stain patterns some distance from the gallery conform to the shape of the gallery from which they originated (Figs. 1, 10).

Economic losses caused by "flag-worm" defects. Hopkins (1894a) estimated that from half to three fourths of the white oak and chestnut oak (*Quercus prinus* L.) in certain areas of West Virginia was affected by the Columbian timber beetle. Current estimates by processors of these species in this area are similarly high. Hay,[1]

[1]Personal correspondence with C. J. Hay, Entomologist, Central States Forest Experiment Station, Berea, Kentucky.

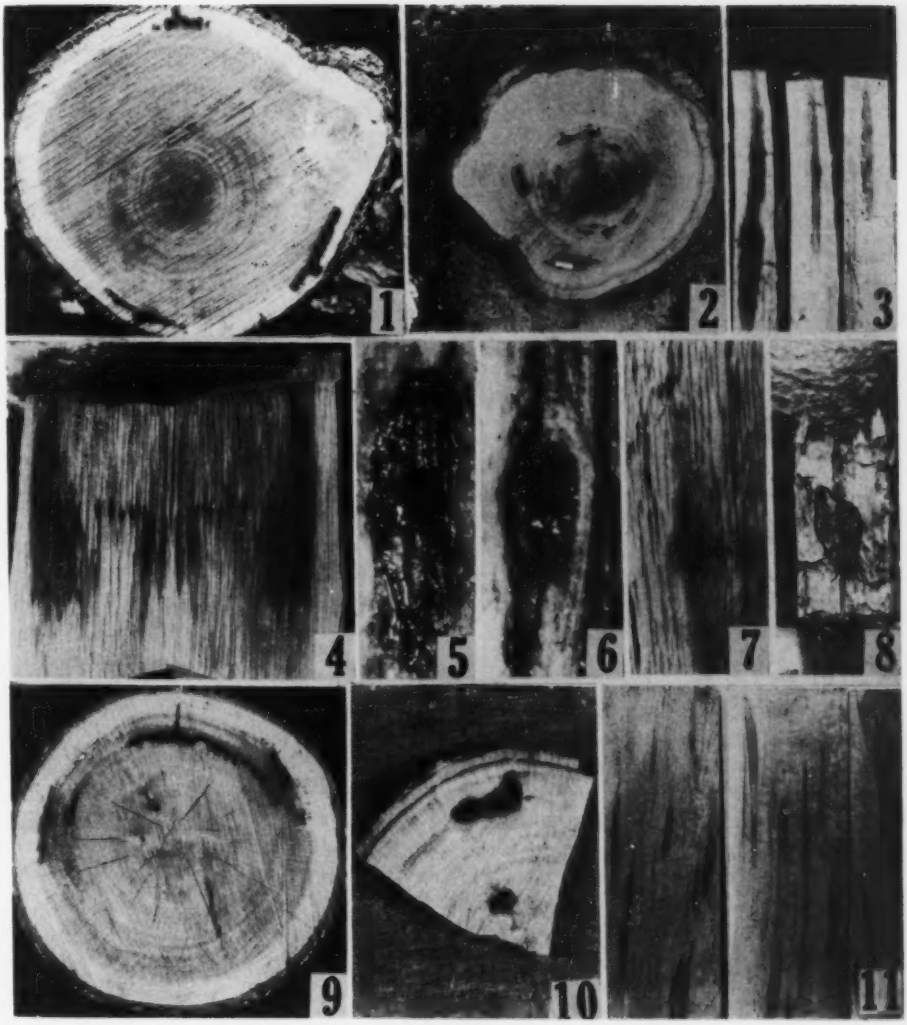

FIGURE 1. *Cross-section of log currently infested.* FIGURE 2. *Stump top of a tree infested a number of years ago.* FIGURE 3. *Current galleries and associated stain. Note that the stains from 2 superimposed galleries are confluent.* FIGURE 4. C. columbianus *"worm holes" and associated stain.* FIGURE 5. *Entrance hole in outer wood. Note slime-flux. Enlarged.* FIGURE 6. *Entrance hole of vacated gallery. Enlarged.* FIGURE 7. *Entrance hole of previous year that has been sealed.* FIGURE 8. *Bark in area of entrance hole. Note "wet stain."* FIGURE 9. *Old gallery sealed in wood.* FIGURE 10. *"Flag" one year after gallery was excavated. Note thicker annual ring year after beetle entered.* FIGURE 11. *Lumber from tree that had been heavily infested by* C. columbianus.

who found 44 percent of the volume of chestnut oak logs in eastern Kentucky damaged by *C. columbianus*, estimated that probably a comparable amount of damage is caused in white oak. Often this damage is the only defect present in wood that would otherwise be perfectly sound.

Buyers of white oak timber have extreme difficulty in predicting the extent to which a particular forest stand may be affected by *C. columbianus*. Often timber is bought at a premium price for veneer logs or stave bolts and later found worthless for the purposes intended because of numerous "flag-worm" defects. The economic losses incurred from such defects vary according to the use made of the wood.

Veneer manufacturers suffer the greatest losses from "flag-worms" because these defects cannot be tolerated in face veneer. One veneer manufacturer in Kentucky[2] has estimated that about 30 percent of his logs bought for veneer purposes at veneer prices have to be used for lumber (a lower value product) due to "worm hole" defects, primary among which are those caused by *C. columbianus*.

Cooperage manufacturers can tolerate only a small number of "flags" in their staves, the number depending upon the location of the defect and the type of cooperage. If confined to the central 4 to 6 inches of the heartwood, the "flags" are not a factor because this portion of the tree is not usually utilized for staves. The stained wood accompanying the galleries of *C. columbianus* and not the galleries themselves cause the greatest difficulty in cooperage manufacturing. Other ambrosia-beetle holes that are not surrounded by extensive stained wood can be filled and thus tolerated. But the "bad or soft wood" associated with the galleries of *C. columbianus* does not hold up under the requirements of tight cooperage.

"Flag-worm" defects also lower the grade of better quality lumber. In the experience of one company,[3] about 20 percent of the lumber from white oak logs that would otherwise fall in a high grade must be downgraded because of excessive worm holes with an attendant loss in value of nearly 40 percent.

All together then damage caused by *C. columbianus* results in much economic loss of timber for veneer, cooperage and lumber.

Anatomy of Corthylus columbianus. Hopkins' (1893) original description of *C. columbianus* is quite brief and incomplete; therefore, a more detailed description is presented. *C. columbianus* is rather stout compared to most members of the *Scolytidae*. Measured specimens averaged 4 mm long and 2 mm wide. The two sexes are the same size, but differ in the size and shape of the antennae, and in the shape and pubescence of the head. The pronotum and elytra are black and the legs reddish-brown. The pronotum (Figs. 12, 13) is 2 mm wide, 2 mm long and it extends hood-like over the head, completely hiding it from dorsal view. It is roughly tuberculated in front, smooth and faintly punctured behind. The elytral punctures are not arranged in distinct rows. The suture of the elytral declivity is elevated and two rows of small tubercles, parallel with the suture, are present on the finely pubescent declivity: there are usually 5 tubercles in each row. Hopkins (1893) pointed out that the presence of small tubercles on the elytral declivity helps distinguish *C. columbianus* from *C. punctatissimus* which is quite similar anatomically. The declivity of the elytra of *C. spinifer* Schwarz is provided on each side with only 3 small tubercles.

The funicle of the antennae of *C. columbianus* has only 1 segment: this is a

[2]Personal correspondence with Dennis Evans, Wood-Mosaic Corporation, Louisville, Kentucky.

[3]Personal correspondence with H. D. Bennett, Appalachian Hardwood Manufacturers, Inc., Cincinnati, Ohio.

characteristic of the genus *Corthylus* (Figs. 12, 13). The club of the antennae is flattened and divided into 3 segments which are separated by 2 sclerotized sutures. These sutures are visible on both sides of the club, but in certain areas they may be less

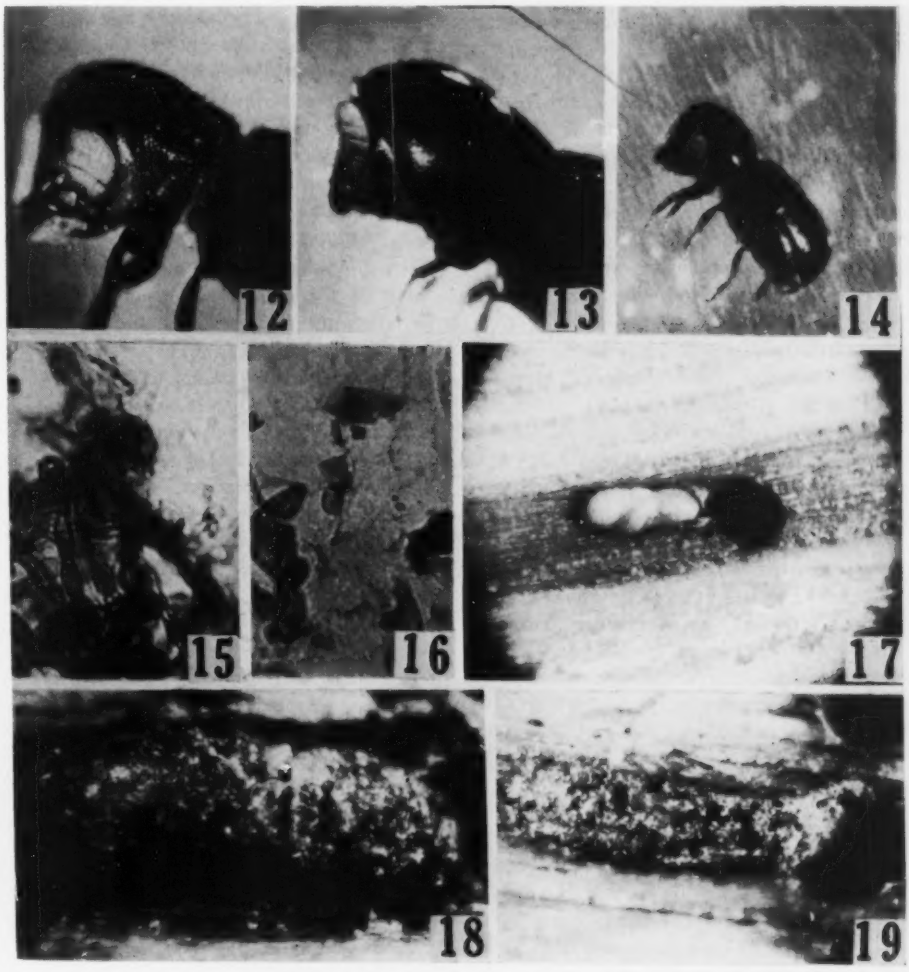

FIGURE 12. *Head and pronotum of female. Enlarged.* FIGURE 13. *Head and pronotum of male. Enlarged.* FIGURE 14. *Adult female. Enlarged.* FIGURE 15. *Section of mycelial "mat" that lines the galleries. Approx. 400 ×.* FIGURE 16. *Mycelial fragments in hind-gut of* C. columbianus. *Approx. 450 ×.* FIGURE 17. *Teneral adult in larval cradle. Note fungus growth plugging entrance to main gallery.* FIGURE 18. *Dead adult beetle found "trapped" for over 150 years in heartwood. Enlarged.* FIGURE 19. *Larval cradle from which beetle in Figure 18 was removed. Note numerous perithecia. Enlarged.*

distinct. The antennae of the female (Fig. 12) are similar in length to those of the male (Fig. 13), but the scape and club are considerably broader. The antennae of the male are not armed with spines as are those of *C. spinifer*.

The frons of the male of *C. columbianus* differs from that of the female in being flattened, finely punctured, and slightly convex in the center (Fig. 13). On the lower margin, near the epistoma, there is a tuft of yellow hair. The frons of the female (Figs. 12, 14) is convex and covered with short, stiff, yellow hairs. The hairs at the margin of the concave indentation of the frons extend beyond those within the indentation and appear as a circular brush when viewed from the front.

The caudal margin of the tibiae of *C. columbianus* is smooth and the anterior margin is serrate. The distal end of the tibiae on the prothoracic leg (Fig. 25) has three rounded teeth and a spine at the distal apex. The distal end of the tibiae on the mesothoracic (Fig. 24) and metathoracic (Fig. 23) tibiae has 4 teeth and a spine on the distal apex. The teeth of the mesothoracic and metathoracic tibiae are more pointed than those of the prothoracic tibiae. The last serration on the anterior margin of the metathoracic tibiae (Fig. 23) is somewhat larger than the others and could be considered to constitute a fifth tooth on the distal end. *Corthylus punctatissimus* is further distinguished from *C. columbianus* in that the middle and hind tibiae of this species have only 3 teeth near the tip.

The sexes of *C. columbianus* were confused when the species was first described by Hopkins (1893, 1894a). Later Hopkins (1894b), after subsequent examination of the genitalia of this species, reversed his descriptions of the male and female. However, no description of the structure of the sexual organs of *C. columbianus* has been published. The genitalia of *C. columbianus* are illustrated (Figs. 20, 22). The intestinal tract of this species is also illustrated (Fig. 21).

Habits of Corthylus columbianus. The life habits of *C. columbianus* are not well known. The beetle enters the stems of vigorous, standing, white oak trees, but the attack is not limited to the tree bole. The galleries of this insect were found also in branches, some as small as 4 inches in diameter. The young adults will commonly reenter the same tree in which they developed to establish a new brood gallery. It is common to find a tree, or a group of trees, that have been reinfested year after year, whereas adjacent trees may be completely devoid of galleries.

Hopkins (1894a) found this beetle within its galleries in white oak on Oct. 7, 1893 but did not describe the stage of development of the galleries. Since he reported finding only a few adults and no eggs, larvae, or pupae, it can probably be assumed that the beetles had completed their development. During the present investigation, a white oak tree, containing both beetles that had just made fresh galleries and others that had completed their development within their galleries, was found on June 28, 1957. From this and from Hopkins' observations it can be concluded that there are at least 2 broods of this beetle per year in white oak. It is not known how the beetles overwinter. Hopkins (1894a) stated that they probably overwinter in galleries excavated in the fall and emerge in the spring to establish new galleries. The trapping of one female beetle in this study on May 25, 1957 indicates that they may begin their activity at least by this date.

Two fungi, *Ceratocystis piceae* and *Pichia* sp. consistently were found associated with *C. columbianus* in its galleries. These fungi were isolated from the galleries and from the beetles. Perithecia of *C. piceae* (Figs. 26, 27) were found in the main galleries and larval cradles of all the current galleries examined. *Pichia* sp. appears to grow primarily in the larval cradles and is readily isolated from the beetles when they are crushed and streaked on nutrient media. Although this fungus is a yeast, its

appearance in the galleries is that of white, fluffy mycelium (Figs. 29, 30). Perithecia similar in size and texture to those of *C. piceae* were found in many of the old galleries that had been sealed in by the growth of the tree. In one case a dead beetle was found in a larval cradle of a gallery that was at least 150 years old (Fig. 18). The larval cradle in which this beetle was located contained numerous perithecia, similar to those of *C. piceae*, that had been preserved for this length of time (Fig. 19). These perithecia were brittle and apparently nonviable as all attempts to culture them failed.

The galleries of *C. columbianus* are always lined with a dense growth of fungus mycelium (Fig. 15). This mycelium is considered to be that of *C. piceae* since in some cases it can be seen to be continuous with perithecia within the gallery. When sterile blocks of wood were inoculated with spores of *C. piceae*, the mycleium produced on and within the wood was similar to the mycelium in the galleries. The mycelium

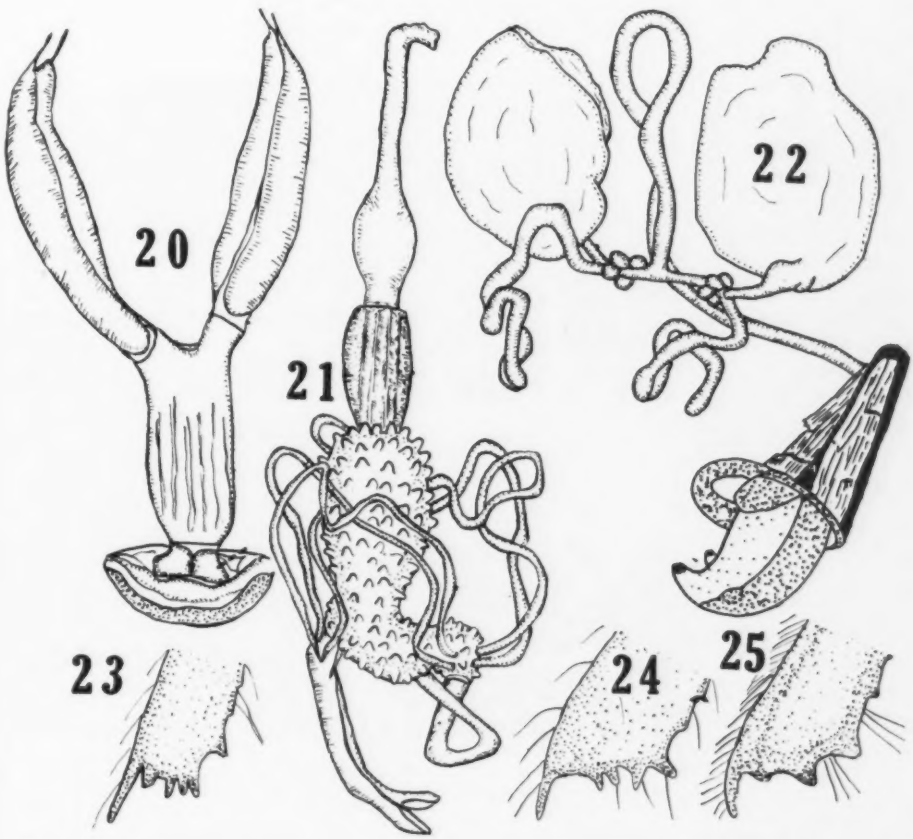

FIGURE 20. *Female genitalia.* FIGURE 21. *Intestinal tract.* FIGURE 22. *Male genitalia.* FIGURE 23. *Distal portion of metathoracic tibia.* FIGURE 24. *Distal portion of mesathoracic tibia.* FIGURE 25. *Distal portion of prothoracic tibia. All greatly enlarged.*

of *C. piceae* that lines the galleries is large and dark colored, giving the gallery a black color (Fig. 9). The bases of the perithecia are usually imbedded in the vessels of the wood and only the necks extend into the gallery (Fig. 26). The necks are kept short by the feeding of the beetles. The conidial (*Graphium*) stage of the fungus was not observed in the galleries. However, when galleries were opened and placed in a moist chamber the *Graphium* stage developed extensively.

On top of the mat of mycelium of *C. piceae* that lines the gallery there is a "slime" composed of fungus spores and bacteria. Adult beetles were observed feeding upon this "slime" and on the necks of the perithecia. Histological studies of the alimentary tract of adult beetles revealed that mycelium, fungus spores and bacteria make up most of the diet of the beetles within the galleries (Fig. 16). Numerous mycelial fragments resembling *C. piceae* were found within the gut of the beetles.

The yeast, *Pichia* sp. was found associated with teneral adult beetles in their larval cradles. Although no larvae were found, it is probable that this fungus con-

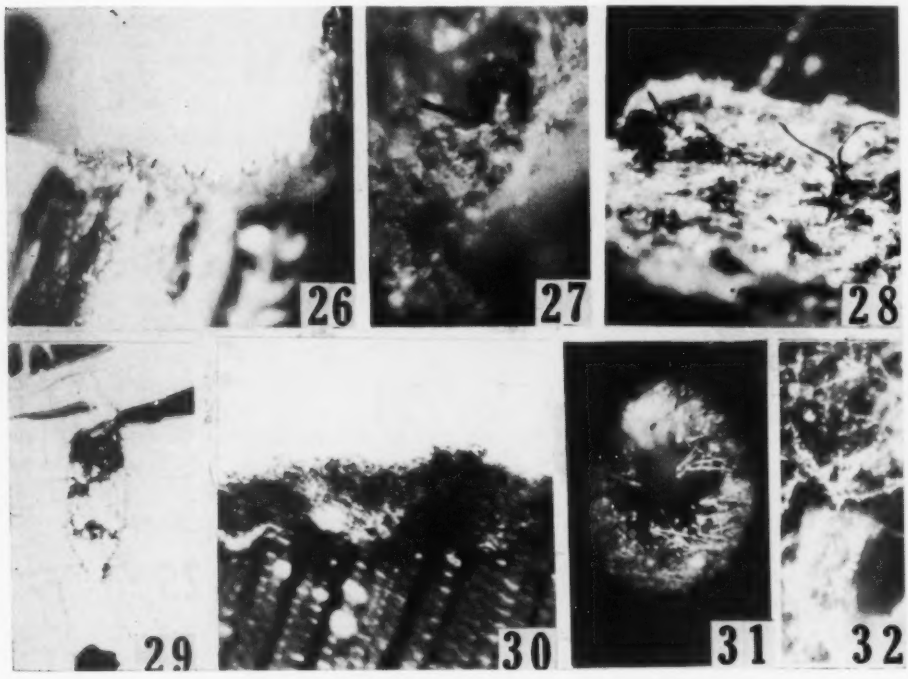

FIGURE 26. *Section of current gallery showing necks of perithecia of* Ceratocystis piceae. *Approx. 30* ×. FIGURE 27. *Perithecium of* C. piceae *in current gallery. Approx. 70* ×. FIGURE 28. *Non-viable perithecia of* C. piceae *in gallery over 150 years old. Approx. 45* ×. FIGURE 29. Pichia *sp. growing in larval cradles of current gallery. Enlarged.* FIGURE 30. *Section of current gallery showing growth of* Pichia *sp. (pseudomycelium). Enlarged.* FIGURE 31. *Old gallery lined with ascophores of* Ascocybe grovesii. *Enlarged.* FIGURE 32. *Conidia of* A. grovesii *in old gallery. Approx. 75* ×.

stitutes a part of their diet. The growth of the yeast in the cradles is subtended by mycelial growth of *C. piceae*. Perithecia of *C. piceae* are usually present within the larval cradles. These two fungi, *C. piceae* and *Pichia* sp., probably constitute the main diet of the larvae and adults because of their consistent presence in the main galleries and larval cradles.

Pichia sp. was commonly found plugging the larval cradles in which there were teneral adult beetles (Fig. 17). This condition would insure both the internal and external contamination of the beetles as they eat their way out of the larval cradle. No definite anatomical adaptation of the beetle could be demonstrated for insuring its contamination by its fungus associates. However, the brush-like structure on the heads of the females (Fig. 14) may be such an adaptation. Yeast cells and fungus spores were found adhering to the hairs of this brush. Sections of the alimentary canal of males and females revealed that the adult beetles feed after emerging from the larval cradles and before leaving the gallery to establish a new brood gallery. The crop and complete intestinal tract of such beetles contained fungus spores, hyphae and bacteria. If the fungus spores are viable after passing through the alimentary canal, the feces could serve as a source of inoculum within a newly established gallery.

A *Fusarium* sp. was often found in current galleries of *C. columbianus* in white oak. This fungus was frequently isolated from the galleries and stained wood and its presence often made it difficult to isolate *C. Piceae* and *Pichia* sp. Examination of scrapings and sections of the galleries revealed that *Fusarium* sp. did not grow or sporulate extensively in the galleries. However, it dominated *C. piceae* and *Pichia* sp. when inoculated in combination with these fungi in pure culture on a malt-yeast extract medium. The cultural characteristics of *C. piceae* have already been adequately described by Hunt (1956). The yeast associated with *C. columbianus* in its larval cradles was placed in the genus *Pichia* according to Wickerham's recent classification (1951). The isolate studied produced hat-shaped ascospores and was unable to assimilate potassium nitrate as a sole source of nitrogen. It required an external source of vitamins for growth and produced both true mycelium and pseudomycelium as well as blastospores. Four ascospores are usually formed in an ascus that deliquesces early.

The nature of the staining. A number of organisms probably contribute to the staining associated with the galleries of *C. columbianus*. *Ceratocystis piceae* is considered to be involved primarily in the staining near the galleries because of the abundance of mycelium of this fungus in this area. This fungus and a species of *Fusarium* both produced similar stains when placed in artificially made holes in white oak trees. Bacteria found in sections of stained tissues may also contribute to the discolorations that develop in the wood.

The hyphae adjacent to the galleries are difficult to trace more than an inch from the gallery as they become lighter in color and are not readily recognized within the protoplasm of the cells. Even when differential staining was employed it was not possible to demonstrate that *C. piceae* or any other fungus extends the full length of the stain.

Except for certain areas of the primary wall of the vessels, only tissues containing parenchymatous cells are discolored by the growth of the fungus. The parenchyma cell membrane is not destroyed when the fungus first penetrates, but later the subsequent growth of the mycelium within the cell destroys the protoplast. The dark hyphae of *C. piceae* may completely fill a parenchyma cell and the cell contents not absorbed by the fungus form a dark matrix around the mycelium. The fungus does not cause a general dissolution of the cell wall and no dissolution of the middle lamella was observed. A partial oxidation of the tannin within the cells appears to occur

as tannin bodies in tissues invaded by the fungus do not show typical reaction when treated with $FeSO_4$. The bodies present in the stained cell are similar in shape, size, and number to the tannin bodies of normal tissues although they fail to give the typical tannin reaction. There is no change in the nature of normal tannin bodies of medullary ray cells adjacent to infected cells and no mycelium was found within medullary cells. This indicates that medullary ray tissue is not attacked by the fungus.

In stained areas there is a partial breakdown of the tyloses in the larger vessels. There appears, in some cases, to be a complete dissolution of the tyloses and in others the tylose walls seem to be ruptured. There does not appear to be enough growth of mycelium within the vessels to account for this condition. Perhaps the invasion of the linear parenchymatous cells adjacent to the vessels causes this breakdown.

A fungus associated with old galleries and stains. Early in this investigation it was found that a fungus (*Ascocybe grovesii* Wells) could be isolated from stained wood associated with old galleries of *C. columbianus*. *A. grovesii* was isolated from wood obtained from areas near Harrisville in Ritchie County and Indian Creek in Wyoming County, West Virginia, representing northwestern and southern areas of the state. *A. grovesii* was isolated from material representing at least 3 different trees indicating that its occurrence in the galleries of *C. columbianus* and accompanying stains may be common.

Some isolations from galleries and stains over 200 years old yielded cultures of this fungus. It was thought at first that it had survived in the wood after the gallery had been closed. However, this fungus was isolated only from trees that had been felled some time previously and ample time had elapsed for external fungi to invade the wood. Isolations from old stains and galleries of freshly cut trees yielded only occasional cultures of bacteria and yeasts and *A. grovesii* was not obtained. This fungus was not found to be associated with *C. columbianus* in its current galleries. Therefore, it is concluded that *A. grovesii* was not introduced into the wood by *C. columbianus* but invades the galleries and stained areas secondarily after the tree has been felled. Davidson and Lombard (1954) isolated *A. grovesii* from the heartwood of red oak and they stated that on this occasion the fungus probably entered the tree after it was felled.

A. grovesii was isolated from stains and galleries but was not obtained when isolations were made from adjacent unstained wood. This fungus grew poorly when placed on sterile blocks of heartwood which further indicates that its growth is confined primarily to the stained wood. It was also observed that certain butt-rot organisms appear to spread more rapidly in the stained areas of wood than in the adjacent bright wood. It was found by a histochemical test that there is an apparent reduction or conversion of the tannins in stained wood which might account for the greater susceptibility of the stained wood to attack by these fungi. Zabel (1948) has presented evidence that indicates a correlation between tannin content of white oak and resistance to certain wood rotting fungi.

The cultural characteristics of *A. grovesii* have been adequately described by Wells (1954). Both the conidial stage (Fig. 32) and the sexual stage (Fig. 31) were found in old galleries and larval chambers. When wood infected by this fungus was placed in a moist chamber numerous conidia and ascospores were produced on the surface of the wood giving it a reddish color.

Discussion

It is recognized that members of the genus *Ceratocystis* are important fungus associates of bark beetles but, as Bakshi (1950) has pointed out, we may have overlooked their importance as associates of ambrosia beetles. Bakshi isolated *Ceratocystis ambrosia* Bakshi

from the galleries of *Trypodendron domesticum* and *Ceratocystis piceae* from the galleries of *T. lineatum*. He thought that the fungus described by Doane and Gilliland (1929) as associated with *Gnathotrichus sulcatus* was probably also *C. piceae*. The finding that *C. piceae* is associated with *C. columbianus* further indicates that species of *Ceratocystis* are important as ambrosia beetle associates. It also points out that *C. piceae* may be an important ambrosia fungus as it has now been reported to be associated with two, and possibly three different ambrosia beetles. The presence of species of *Ceratocystis* as associates of ambrosia beetles reveals a striking parallel between the insect-fungus relations of ambrosia beetles and bark beetles.

Webb (1945) expressed the opinion that all ambrosia fungi from different parts of the world may belong to the genus *Leptographium*. Bakshi (1950) in subsequent work did not find this to be the case and the present investigation also failed to confirm Webb's concept. It is reasonable to expect that in the course of evolution a variety of fungi have become associated with both ambrosia beetles and bark beetles.

Since *C. columbianus* apparently attacks vigorous trees, it could be an effective vector of such a wilt-pathogen as *Ceratocystis fagacearum* (Bretz) Hunt, which must enter fresh wounds in order to become established in the tree. Even if *C. columbianus* does not serve directly as a vector of such a pathogen, its entry holes in the wood could provide an infection court for pathogens carried by other insects found to enter its galleries. But, the fact that *C. columbianus* is localized in its distribution and frequently reinfests the tree in which it developed, limits its potential as an efficient vector of a wilt-disease pathogen. Also because this insect is a native species with a long standing association with its ambrosia fungi, it may be less apt to become assocated with other fungi. The fact that many trees were found that were still in a vigorous condition after having been abundantly infested over many years by this beetle would indicate that at present *C. columbianus* is not associated with a "killing" pathogen. Nevertheless, the wounds that are made when the beetles enter living trees should not be overlooked as possible infection courts for such pathogens.

It was recognized early by Hopkins (1894a) that there was no means or need to control *C. columbianus* directly, since most of the damage is done to the tree many years before the tree is utilized. However, several possible steps might be taken to reduce the losses that result from this pest. An understanding of the ecological factors that govern the distribution of the insect might make possible an intelligent selection of sites or trees likely to be relatively unaffected by it. Information on the environmental conditions that govern the distribution of *C. columbianus* would also be useful in later years when there will probably be more extensive planting of hardwood species. Such information could be used to select planting sites for white oak seedlings that would be unfavorable for the development of the beetle.

The damage caused by the galleries of *C. columbianus* and accompanying stains makes affected white oak wood unsuitable for many specialized high value uses. However, Hopkins (1894a) has pointed out that the presence of the beetle holes and "Columbian stains," in some cases, enhances the aesthetic beauty of the wood. He predicted that a demand might develop for the presence of these "defects" in furniture and other manufactured goods. Columbian beetle defects cannot be tolerated in veneer where they interfere with the assembly of this extremely valuable product, but there appears to be no reason why solid paneling for the interior of buildings could not be manufactured from affected material. Since the public now accepts knotty pine, pecky cypress and even woods affected by incipient white pocket rot as decorative, "Columbian stains" should also be accepted for special purposes.

Summary

It is estimated that 50 to 75 percent of the white oak trees in certain areas of West Virginia are affected by the Columbian timber beetle. Processors of white oak in this area, particularly veneer and cooperage manufacturers, consider *C. columbianus* to be their most destructive economic pest. The stained wood accompanying the galleries of the beetle is more extensive than that associated with other ambrosia beetle galleries, and it is the "flags" of stained wood and not the galleries themselves that cause the greatest damage. "Flag worm" defects cannot be tolerated in face veneer and only a small number of "flags" are permissible in cooperage stock. Such defects also seriously affect the grade of better quality lumber.

Only one standing oak tree was located that was currently infested by *C. columbianus*. This tree presented no signs of infestation until felled, when "flags" were observed in cross sections of the bole. The "wet stains" mentioned by Hopkins were apparent, but only when the tree was closely examined after felling. The stains associated with the galleries uusally extend an equal distance above and below the galleries and appear to reach their maximum length the year the galleries are made.

The sexes of *C. columbianus* were confused when the species was first described. Although Hopkins later reversed his original descriptions, some confusion still exists in the literature. Therefore a description of the secondary sexual characters is presented.

Two fungi, *Ceratocystis piceae* and *Pichia* sp., were found consistently associated with *C. columbianus* in its galleries. These fungi and associated bacteria appear to constitute most of the diet of the adult beetles within the galleries. Perithecia of *C. piceae* were found in the main galleries and larval cradles of all the current galleries examined. *Pichia* sp. appears to grow primarily in the larval cradles and was readily isolated from the beetles when they were crushed and streaked on nutrient media. Perithecia similar in size and texture to those of *C. piceae* were found in many of the old galleries that had been sealed by the growth of the tree. One case was found where both perithecia and an adult beetle were sealed in the tree in a gallery over 150 years old.

A *Fusarium* sp. was also frequently isolated from the current galleries and stained wood and its presence made it difficult to isolate *C. piceae* and *Pichia* sp. Both *Fusarium* sp. and *C. piceae* produced indistinguishable stains when placed in artificially made holes in standing white oak trees. Isolation from old galleries and stains in trees that had been felled for some time yielded cultures of *Ascocybe grovesii*. This fungus is considered a secondary invader of the galleries and stained wood and probably gains access to the wood through cut surfaces after the tree is felled. It is thought that the original staining organisms predispose the wood so that it is more favorable for the growth of other fungi.

The fact that *C. piceae* is a fungus associate of *C. columbianus* further indicates that species of *Ceratocystis* may be more important as food for ambrosia beetles than originally thought. It also points out the similarity of the fungus associations of ambrosia beetles to those of bark beetles which commonly have species of *Ceratocystis* in their galleries. The bark beetles and ambrosia beetles then are not separable on the basis of any recognized difference in their insect-fungus relations but on the basis of their feeding and breeding location within the tree.

The wounds made by the entrance of *C. columbianus* into living white oak trees are recognized as possible infection courts for pathogenic fungi. But, since this beetle appears to be localized in its distribution within a stand and commonly reinfests the same tree in which it developed, its potential as an efficient vector of a pathogenic fungus may be limited. It is suggested that studies

of the factors that govern the distribution of *C. columbianus* might yield information that could be used to select trees relatively free of the galleries of this beetle.

Literature Cited

ANONYMOUS. 1956. Southern Forest Insect and Disease Newsletter. No. 2. U. S. Forest Service, Southern For. Expt. Sta.

————. 1957. Report of Virginia Economic Insect Detection and Reporting Service. September.

BAKSHI, B. K. 1950. Fungi associated with ambrosia beetles in Great Britain. Trans. Brit. Mycol. Soc. 33: 111-120.

DAVIDSON, R. W. and F. LOMBARD. 1954. Brick red stain of sitka spruce and other wood substrata. Phytopathology 44: 606-607.

DOANE, R. W. and O. J. GILLILAND. 1929. Three California ambrosia beetles. Econ. Ent. 22: 915-921.

HOPKINS, A. D. 1893. Notes on the discovery of a new scolytid, with brief description of the species. Proc. Ent. Soc. Wash. 3: 104-105.

————. 1894a. Black holes in wood. W. Va. Agric. Expt. Sta. Bull. 36.

————. 1894b. Sexual characters in Scolytidae. Can. Entomologist 26: 274-280.

————. 1903. Some of the principal insect enemies of coniferous forests in the United States. Yearbook, U. S. Dept. of Agric. pp. 265-282.

HUBBARD, H. G. 1897. The ambrosia beetles of the United States. U. S. Dept. of Agric. Div. Ent. Bull. 7.

HUNT, J. 1956. Taxonomy of the genus Ceratocystis. Lloydia 19: 1-59.

LOCKARD, C. R., PUTNAM, J. A. and R. D. CARPENTER. 1950. Log defects in Southern hardwoods. U. S. Dept. Agric. Handbook No. 4.

WEBB, S. 1945. Australian ambrosia fungi. Proc. Roy. Soc. Victoria 57: 57-78.

WELLS, D. E. 1954. Ascocybe, a new genus of lower ascomycetes. Mycologia 46: 37-51.

WICKERHAM, L. J. 1951. Taxonomy of yeasts. U. S. Dept. Agric. Tech. Bull. 1029.

ZABEL, R. A. 1948. Variations in the decay resistance of white oak. New York Coll. of For. Tech. Pub. 68.

Absorption Rates of 2,4,5-T Tagged with Carbon-14

BY
LAURENCE C. WALKER
EDWIN BECK
ERWIN DUMBROFF

THE SYNTHETIC HORMONE, 2, 4, 5-T, is widely used in forest, utility, and agricultural industries for controlling undesirable trees, brush, and herbs. Various formulations are prepared by chemical laboratories and offered on the market. The rapid withdrawal and replacement of marketed products has resulted, in large part, from inconsistent effects on treated vegetation. Often, perhaps unwisely, these inconsistencies have been attributed to chemical factors and species differences rather than to site and weather factors.

The present research aims to define more specifically the relative intake of three formulations in water oak (*Quercus nigra* L.) and sweetgum (*Liquidambar styraciflua* L.). Acetone, triethylamine salt, and ammonium salt solutions of 2, 4, 5-T were used in this initial study because of their potential industrial use in weed-tree control. Further studies will deal with esters, currently extensively employed in silvicultural operations.

When tagged with radioactive carbon-14 (C^{14}) in the carboxyl group, the growth regulator 2, 4, 5-trichlorophenoxyacetic acid may be quantitatively analyzed in plant tissues. Ordinarily, quantitative analyses are made by measuring chlorine content of a sample, but when dealing with plant tissues this is an ineffective procedure. Not only are numerous chlorine compounds present in plant organs, anthocyanin being a notable example, but also the minute quantities of 2, 4, 5-T translocated to plant parts dictate the need for a more sensitive and efficient technique.

Literature Review

Early work with radioactive growth regulators dealt with 2, 4-dichlorophenoxyacetic acid and 3-iodo-3-nitrobenzoic acid tagged with iodine-131 (Wood *et al.* 1947; Mitchell *et al.* 1947; Mitchell and Linder, 1950; Linder *et al.* 1950; Mitchell and Linder, 1950a; Gallup and Gustafson, 1952; Bryon and Fuller, 1952). The radio element in the case of 2, 4-D was attached to, but was not a part of, the growth regulator molecule. Consequently it was difficult to establish to what extent the absorption and translocation of the phenoxy compound was affected. With the

---

L. C. Walker and E. Beck are Associate Professors of Silviculture and Botany, respectively, at the University of Georgia, Athens. E. Dumbroff, formerly a graduate research fellow, is now at the East Gulfport Research Center, Southern Forest Experiment Station. This is a contribution of the Forest Physiology Laboratory, University of Georgia, jointly financed with the Georgia Forest Research Council and the Georgia Forestry Commission. Harold Morris provided valuable consultation. This paper is Journal Series No. 74 of the College Experiment Station.

synthesis of 2, 4-D in which one or more of the carbon-12 (C^{12}) atoms were substituted by their radioactive C^{14} isotope, critical evaluation could be made of absorption, translocation, and metabolism. In 1953 Mitchell *et al.* employed 2, 4-D labelled with C^{14} to show that boron and sucrose solutions accelerated movement of phenoxy acid.

Holley *et al.* (1950) used 2, 4-D labeled with C^{14} in the carboxyl group. Highest concentrations of radioactive carbon were found in the petiole six hours after treatment of primary leaves. The first internode and the hypocotyl were next in concentration. They reported that over one-half of isolated C^{14} was not in the 2, 4-D molecule. Further, the amount of 2, 4-D absorbed varied from 25 to 60 percent, even though application and environmental factors were controlled. As a follow-up to the work of Holley and his colleagues, Fang *et al.* (1951) employed 2, 4-D labeled with C^{14} in the methylene group. Following application to bean leaves, they found greatest accumulations of radioactivity in stems. Very little radioactive carbon was isolated in untreated leaves and roots. Stem activity increased with a lapse of time up to 144 hours after treatment. Rate of absorption of 2, 4-D was greatest for young bean plants, but no appreciable differences were apparent between 50 and 150 microgram treatments. With the alpha-methylene tag, these workers were able to determine that 17 percent of the radioactive chemical applied was released to the atmosphere as $C^{14}O_2$. Weintraub *et al.* (1952) in a further study of the transformation of C^{14}-labeled 2, 4-D to $C^{14}O_2$ tagged the growth regulator on the phenoxy ring, in the methylene group, and in the carboxyl group. During the first 24 hours, a much greater fraction of the carboxyl carbon than of the methylene carbon was converted to CO_2. Subsequently, the rate of evolution from carboxyl diminished while that from methylene increased. Increasing the dosage increases the amount of carbon evolved as $C^{14}O_2$, but diminishes the fraction of the total dose that is converted. Because of low activities of ring-labeled 2, 4-D, higher doses than with chain-labeled compounds are necessary, since only negligible amounts of the latter were converted and liberated as CO_2 in bean plant experiments.

Weintraub *et al.* (1954) also studied carboxyl-, methylene-, and ring-labeled 2, 4-D. When applied to buds of *Prunus avium*, 20 percent of the chemical did not penetrate the bud at the end of one week, regardless of dosage form. They reported further that a small amount of carbon in 2, 4-D was transformed to other products.

Crafts' (1956, 1956a) and Leonard and Crafts' (1956) work with 2, 4-D labeled with C^{14} dealt specifically with beans, morning glory and California species of shrubs. Using autoradiographs of quick-frozen tissue, the tracer was found well distributed throughout the petiole and treated leaf, stem, and generally in the root system. Occasional movement was traced to petioles and main veins of leaves opposite treated ones, but never were high concentrations found in terminal buds. On the other hand, analysis of killed tissues showed that considerable C^{14} had moved to terminal buds. In reporting on the elapsed time for 2, 4-D to reach various organs it was noted that the chemical was found in roots after five hours. Plants growing in a moist condition retained 2, 4-D in solution for a much longer time than those in unsaturated open air.

Blue oak (*Quercus douglasii*) treated with tagged 2, 4-D reacted similarly to field treatments with stable 2, 4-D. Leaves of this species are apparently very sensitive to this chemical and, as a result, necrosis occurs before translocation. Tracer studies were negative. In contrast, in live oak (*Q. wislizenii*), translocation of tagged 2, 4-D was active from February through September. Except for a period in March after new growth started, movement was principally downward (Leonard and

Crafts, 1956). These authors emphasized the reduction of absorption caused by contact injury of silvicides and noted the importance of adequate soil moisture, active growth, and its accompanying food movement in translocation.

Methods

Two microcuries of radioactivity of triethylamine salt, ammonium salt, and acetone formulations of tagged 2, 4, 5-T were applied as droplets on upper leaf surfaces. This activity represents 7.386 milligrams of 2, 4, 5-T in 100 lambdas of solution. Seedlings of water oak two years old and 2 feet high, and of sweetgum one year old and 1 foot high were treated separately in greenhouse experiments. The statistical design was a split-split plot with whole plots completely randomized for water oak and a split for sweetgum tests. Twelve leaves on each water oak, six in the uppermost whorl and six near the midpoint, were randomly selected. For sweetgum, position was not recorded and only six leaves were sampled. Three replicate trees for each treatment were employed. Two leaves at each position from each tree were collected at intervals of one day, one week, and one month following treatment. In addition to treated leaves, untreated leaves, stems, and roots from these plants were sampled at the end of 30 days.

Extraction of the radioactive 2, 4, 5-T from plant tissues with hot 80 percent ethyl alcohol and preparation of the extracted material for disintegration counting followed the procedure presented in detail by Beck and Walker (1958). Treated leaves were first washed with cold 80 percent ethyl alcohol to remove unabsorbed 2, 4, 5-T.

The extracting procedure follows:

Place tissue in 100 ml boiling 80 percent ethyl alcohol and continue to boil 3 minutes. Blend in Waring blendor and centrifuge at ca 2000 rpm for 2 minutes or until residue is separated. Re-extract residue twice with 40 ml of hot 80 percent alcohol. Then combine supernatants of extracts, distill to ¼ volume, cool to room temperature, alkalize to pH 7.5 with *N* NH_4OH, and centrifuge. Decant the supernatant, wash the residue twice with *N* NH_4OH, centrifuge, combine the supernatants and make to 100 ml volume with 80 percent alcohol. Dry 1 ml aliquot evenly on a stainless steel planchet under an infrared light, cool, and determine the disintegration rate by a gas-flow Geiger-Müller counter. Stress careful drying to obtain a good geometrical pattern of the sample. Average two counts of duplicate samples for each leaf.

Paper chromatographs were employed in qualitative identification of 2, 4, 5-T for assurance that the extracted materials were not converted to other compounds as discussed by Hay (1956) and shown by Weintraub *et al.* (1954) for ring-labeled 2, 4-D. With a solvent system of the non-aqueous phase of iso amyl alcohol, NH_4OH, centrifuge, combine the supernatants mended by Siegelt and Schlögl (1955) for chlorinated phenols and phenoxyacetic acids, the Rf values of 2, 4-D and 2, 4, 5-T were established colorimetrically between 0.3 and 0.4. Spots of extracts containing the labeled isotope are readily counted at these Rf values. The Siegelt and Schlögl solution was used because the Rf values are intermediate whereas these values are high for phenoxy compounds in most solvents. Using 0.5 ml spots (from the 100 ml volumetric extracts) on Whatman No. 1 filter paper, C^{14} was counted using the gas-flow windowless G-M counter.

The ammonium and triethylamine salt formulations were synthesized by (1) diluting 1 part of pure radioactive 2, 4, 5-T with 9 parts of pure non-radioactive chemical, (2) dissolving with a small amount of 95 percent ethyl alcohol, (3) adding an excess of ammonium hydroxide or triethylamine to make the ammonium salt or the triethylamine salt, respectively, (4) drying in a vacuum oven, (5) dissolving in water, (6) adding 0.05 ml of a wetting agent (Tergitol anionic 7), and (7) making to 10 ml volume in 95 percent alcohol. The

acetone solution was produced as in steps (1) and (6) above, and made to volume in acetone. After exterior chemical was washed from surfaces with alcohol, autoradiograms of leaves were made by placing in contact with X-ray film for 30 days.

In the interest of finding a method of C^{14} analysis for use where refined G-M counters and scalers are not available, five lambda drops of extracted material were placed on paper, and then exposed to X-ray film. The films were developed after refrigerating for periods of 6 hours to 30 days, but quantitative differences could not be estimated for the different periods.

Results and Discussion

Water oak. Triethylamine salt was absorbed in greater quantities than either ammonium salt or acetone solution of 2, 4, 5-T and ammonium salt was least (Table 1, Figs. 1 and 2). Both acetone and ammonium salt solutions left white flaky films on leaf surfaces, while triethylamine salt residues were invisible. Interestingly, the superiority of triethylamine substantiates the contention of one of the major utility line contractors that better kill of brush is obtained with amine formulations. Amounts absorbed during the time between treatment and sampling—from one to 30 days—did not vary significantly for the three formulations combined, although for triethylamine salt alone, statistical significance fell just below the 5 percent level. Rates of absorption did not differ between upper and lower leaves.

Water oak leaves have no stomates on their dorsal surfaces. Since the solutions of silvicide were applied to these surfaces, it is possible that superior penetration of triethylamine was due to a reaction between constituents of the cuticle and this radicle. The cuticle, among other things, consists of a number of modified fatty acids which react with amines. This may account for better penetration of oak leaves by amine salt than by acetone solution or ammonium salt.

Much excess 2, 4, 5-T was washed from treated leaf surfaces at sampling time prior to analysis. Data in Table 1 show a

TABLE 1. Relative quantity of 2, 4, 5-T absorbed by water oak leaves at two positions, and that obtained from leaf washings, as measured by disintegration rates of radioactive carbon-14 for various periods of elapsed time.

Chemical and position	1 day		1 week		1 month		Average	
	In leaves	From washings	In leaves	From washings	In leaves	From washings	In leaves	From washings
	counts per minute							
Acetone solution								
High	632	122	424	528	634	738	563	462
Low	578	516	470	245	752	600	600	453
Average	605	319	447	386	693	669	582	458
Triethylamine salt								
High	1422	515	1834	469	2363	281	1873	422
Low	1313	768	2447	393	2862	285	2207	482
Average	1367	641	2140	431	2612	283	2040	452
Ammonium salt								
High	105	284	27	233	79	201	70	239
Low	162	223	41	245	83	408	95	292
Average	134	254	34	239	81	304	83	266

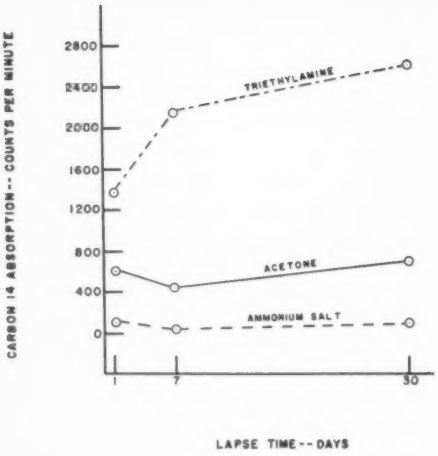

FIGURE 1. *Rates of absorption of three formulations of 2, 4, 5-T applied to water oak.*

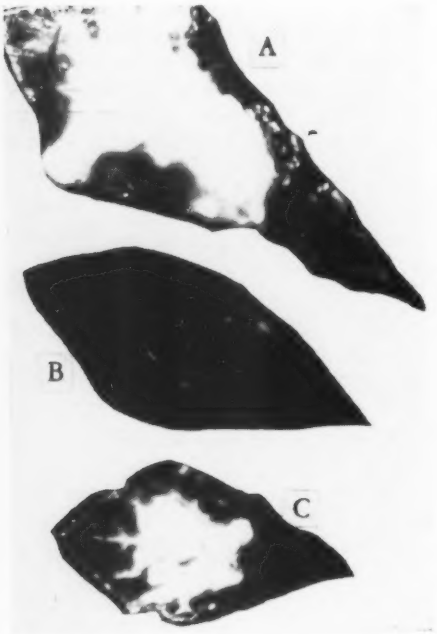

FIGURE 2. *Autoradiogram of treated water oak leaves 30 days after treatment. A. triethylamine; B. ammonium; C. acetone.*

lack of a relation between absorption and washings. The excess chemical on leaves treated with acetone and triethylamine was about the same, but very little 2, 4, 5-T was washed from leaves treated with the ammonium salt. This suggests that material neither in the leaves nor washed from them at time of sampling might have been previously translocated to other plant parts. However, dilution of 2, 4, 5-T was apparently so great at the end of one month as to render the radiation counts for stems, roots, and untreated leaves insignificant above the background. The data in Table 1, indicating an increase of 2, 4, 5-T on leaf surfaces with time when treated with acetone and ammonium salt preparations illustrate, of course, an improbability. However, these disintegration counts are within the limits of experimental error.

In practice, apparently, large portions of silvicides that are left on leaves are washed off by rain or remain to decompose with the leaf. Knowledge of the saturation point for various formulations should effectively reduce cost of control operations.

The lower order error terms were statistically highly significant when tested against highest order sampling error, indicating that something other than sampling error is entering into experimental error. Perhaps errors due to imperfect selection, bias in response, variation in rate of isotope disintegration, and heterogeneity of sample geometry are responsible (Table 2).

Most of the C^{14} has been retained in the 2, 4, 5-T molecule, and has not been chemically exchanged (Table 3). The Rf value of 0.35 at which the growth regulator was mapped holds for these formulations (Fig. 3). It is possible that a minute amount of radioactive carbon is exchanged with the C^{12} of other compounds or occurs in the degradation products of 2, 4, 5-T. These compounds were counted at an Rf value of 0.2 principally. Minute amounts of non 2, 4, 5-T compounds were recorded at 0.1, 0.5, 0.8, and 0.9 values. The Rf values were similar for all treat-

ments, but concentrations were considerably less for the ammonium salt than for others.

The weakness of the beta particle emitted by C^{14} (six inches of air will shield its radiation) means that large doses in plant tissues are necessary in order for some atoms to pass through the shielding effect of those tissues. But large doses kill tissues before translocation is complete. Autoradiograms of untreated plant parts exposed to X-ray film for periods up to two months revealed only negligible movement to those parts or accumulation in them.

At one day after treatment, leaves treated with triethylamine were necrotic and other (untreated) leaves on trees so treated were chlorotic. Leaves on plants treated with acetone were moribund. Ammonium-treated leaves were unaffected. One week later, untreated leaves of the triethylamine-treated plants were quite chlorotic; and a few untreated leaves of the acetone plants were necrotic. With the ammonium salt, some untreated leaves were moribund, but treated leaves remained healthy in appearance. This situation persisted, and appears to substantiate the generally accepted hypothesis that death of leaf tissues inhibits absorption and subsequent translocation and, therefore, is responsible for poor kill of vegetation treated with 2, 4, 5-T.

The depression in absorption of 2, 4, 5-T at the second sampling period for the acetone and ammonium salt treatments

TABLE 3. Average disintegration rates of all treatments of water oaks for chromatographic strips.

Rf[1]	Counts per minute of radioactivity
0.1	Trace
0.2	17
0.3	99
0.4	92
0.5	Trace
0.6	—
0.7	—
0.8	Trace
0.9	Trace
1.0	

[1]The fraction of the total distance through which the capillary stream carried tagged 2, 4, 5-T.

TABLE 2. Analysis of variance summary.

	All treatments				Triethylamine salt treatments	
	Inside leaf extracts		Outside leaf washings		Inside leaf extracts	Outside leaf washings
	Water oak	Sweetgum	Water oak	Sweetgum	Water oak	
Treatment	0.01	0.01	0.05	0.01	—	—
Replications	ns	ns	ns	ns	ns	ns
Position	ns	—	ns	—	ns	ns
Date	ns	0.01	ns	0.01	ns	ns
Position × treatment interaction	ns	—	ns	—	—	—
Date × treatment interaction	0.05	0.01	0.01	0.01	—	—
Date × position interaction	ns	—	ns	—	ns	ns
Date × position × treatment interaction	ns	—	ns	—	—	—
Experimental error	0.01	ns	ns	ns	0.01	ns

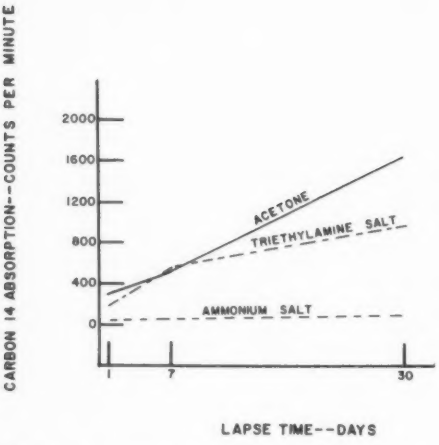

FIGURE 4. *Rates of absorption of three formulations of 2, 4, 5-T applied to sweetgum.*

suggests that chemical moves out of the leaf at a more rapid rate than it enters for a short period before tissues die. By the third sampling period, necrosis prevents outward physiological translocation to other plant tissues, but molecular diffusion into leaves from outer leaf surfaces continues. For triethylamine salt, outward translocation is probably interrupted by early necrosis.

Sweetgum. The response of sweetgum to the 2, 4, 5-T treatments differed from water oak, notably, in the relationship between elapsed time and the amount of chemical found in treated leaves. For all treatments combined, the amount absorbed increased with time at a rate which was statistically highly significant; but for ammonium salt alone, no real differences were apparent.

Quite in contrast to the water oak results, acetone was more readily absorbed than triethylamine salt of 2, 4, 5-T, except at the one week period. Ammonium salt had the lowest concentration; and in this respect, the sweetgum behaved as did the water oak. Treatment differences were significant at the 1 percent level (Figs. 4, 5; Table 4).

Sweetgum has stomates on both leaf surfaces. More rapid absorption of acetone may be due to its low surface tension, in contrast to the water solutions of the salts, thereby permitting penetration more readily

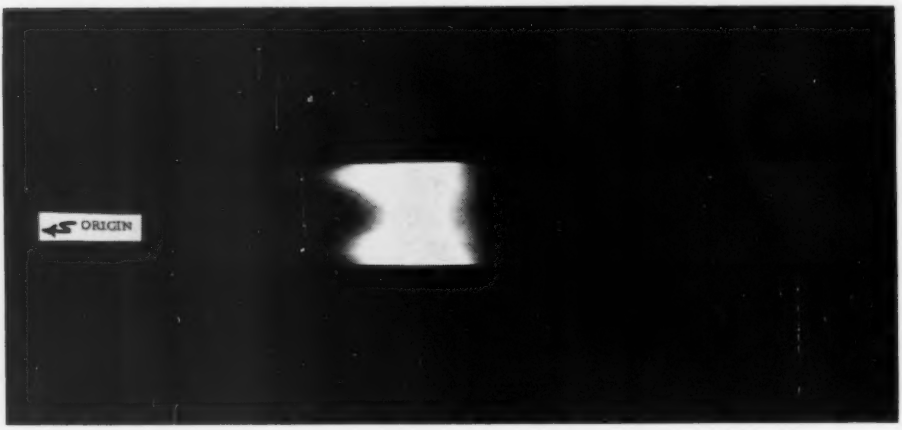

FIGURE 3. *Autoradiogram of chromatogram showing relative distribution of C^{14}. The sunburst at an Rf of about 0.35 is 2, 4, 5-T.*

through stomates on the upper surface.

For all sweetgum treatments, the amount of 2, 4, 5-T in the leaves and the amount washed off was not inversely related as would be expected. However, a close relationship exists between the extracted material and the excess washings: i.e., the greater the concentration within the leaf, the greater the amount washed off (Table 4). As previously theorized, this may be an indication that tissues showing low analysis may have high absorption rates; and that the absorbed material had been translocated to other plant parts prior to analysis. As shown in Table 2, treatment, date, and treatment-date interaction were highly significant for 2, 4, 5-T in leaves at time of sampling.

Leaf position was ignored in the sweetgum experiment because of the lack of significance in the previously-analyzed water oak data and because the sweetgum seedlings available did not lend themselves conveniently to such a sampling procedure (no whorls, too few leaves, and too little variation in height of low and high leaves).

As was the case with water oak, triethylamine salt showed signs of affecting the plants within a day after treatment. At the third sampling period, all trees were still alive; but at 8 weeks after treatment all triethylamine- and acetone-treated sweetgum were dead. Ammonium salt, however, did not adversely affect sweetgum at this late date.

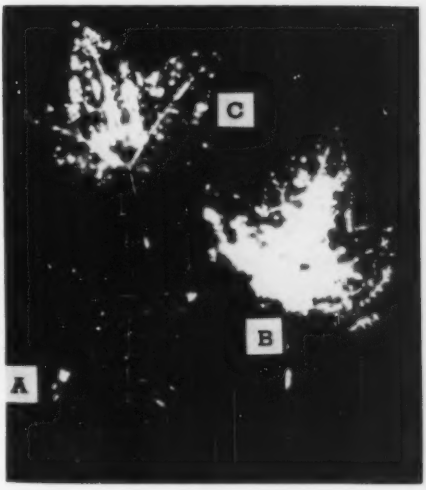

FIGURE 5. *Autoradiogram of treated sweetgum leaves 30 days after treatment. A. ammonium; B. acetone; C. triethylamine.*

Summary

Employing 2, 4, 5-T tagged with C^{14} as a simulated spray on water oak and sweetgum seedlings, absorption of three formulations was compared. For water oak, triethylamine salt was highly superior, followed by acetone solution and ammonium salt. For sweetgum, acetone was most readily absorbed, followed by triethylamine and ammonium salts. Absorption did not increase after one day for water oak, nor

TABLE 4. Relative quantity of 2, 4, 5-T absorbed by sweetgum leaves, and that obtained from leaf washings, as measured by disintegration rates of radioactive carbon-14 for various periods of elapsed time.

Chemical	1 day		1 week		1 month		Average	
	In leaves	From washings	In leaves	From washings	In leaves	From washings	In leaves	From washings
	Counts per minute							
Acetone	306	7674	522	5305	1655	1923	828	4967
Triethylamine	180	2954	560	1240	986	1314	575	1836
Ammonium	49	750	55	633	105	1135	70	839

did the rate differ with elevation of treated leaves. Concentrations within leaves of sweetgum, on the other hand, increased with elapsed time between treatment and sampling. Very little C^{14} in the 2, 4, 5-T was transferred to other compounds, according to chromatographic tests.

Literature Cited

BECK, E. G., AND L. C. WALKER. 1958. Extraction of C^{14}-tagged 2, 4-D and 2, 4, 5-T from woody plants. Proc. So. Weed Conf.

BYRON, B. O., AND W. H. FULLER. 1952. Translocation of 2, 4-dichloro- 5-iodophenoxyacetic acid in velvet mesquite seedlings. Bot. Gaz. 113: 368-372.

CRAFTS, A. S. 1956. Translocation of herbicides. I. The Mechanism of translocation: Methods of study with C^{14}-labeled 2, 4-D. Hilgardia 26: 287-334.

———. 1956a. Translocation of herbicides. II. Absorption and translocation of 2, 4-D by wild morning-glory. Hilgardia 26: 335-365.

FANG, S. C., E. G. JAWORSKI, A. V. LOGAN, V. H. FREED, AND J. S. BUTTS. 1951. The absorption of radioactive 2, 4-dichlorophenoxyacetic acid and the translocation of C^{14} by bean plants. Arch. Biochem. & Biophys. 32: 249-255.

GALLUP, A. H., AND F. G. GUSTAFSON. 1952. Absorption and Translocation of radioactive 2, 4-dichloro-5-iodo-131-phenoxyacetic acid by green plants. Plant Physiol. 27: 602-612.

HAY, J. R. 1956. Present status of translocation investigations. Proceedings Ninth Annual Meeting, Western Section, National Weed Committee 20-22.

HOLLEY, R. W., F. B. BOYLE, AND D. B. HAND. 1950. Studies of the fate of radioactive 2, 4-dichlorophenoxyacetic acid in bean plants. Arch. Biochem. 27: 143-148.

LEONARD, O. A., AND A. S. CRAFTS. 1956. Translocation of herbicides. III. Uptake and distribution of radioactive 2, 4-D by brush species. Hilgardia 26: 366-415.

LINDER, P. J., J. W. MITCHELL, AND J. W. WOOD. 1950. Effect of 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives as plant growth regulators. Sci. III: 518-519.

MITCHELL, J. W., W. M. DUGGER, AND H. G. GAUCH. 1953. Increased translocation of plant growth modifying substances due to applications of boron. Sci. 118: 354.

MITCHELL, J. W., AND P. J. LINDER. 1950. Absorption of radioactive 2, 4-DI as affected by wetting agents. Proc. NE Weed Control Conf. 21-25.

MITCHELL, J. W., AND P. J. LINDER. 1950a. Absorption and translocation of radioactive 2, 4-DI by bean plants as affected by cosolvents and surface agents. Sci. 112: 54-55.

MITCHELL, J. W., AND P. J. LINDER. 1957. Absorption and translocation of plant regulating compounds. In Atomic Energy and Agriculture. Publ. 49 AAAS: 165-182.

MITCHELL, J. W., J. W. WOOD, W. C. WOLFE, AND G. W. IRVING, JR. 1947. Relative growth rates of bean and oat plants containing known amounts of a labeled plant-growth regulator (2-iodo-131-3-nitrobenzoic acid). Science 106: 395-397.

SIEGELT, A., AND K. SCHLÖGL. 1953. The separation by paper chromatography of chlorinated phenols and chlorophenoxyacetic acids. Monatsh. 84: 686-689. (Chem. Abstr. 1954: 3204H).

WEINTRAUB, R. L., J. W. BROWN, M. FIELDS, AND J. ROHAN. 1952. Metabolism of 2, 4-dichlorophenoxyacetic acid. I. $C^{14}O_2$ production by bean plants treated with labeled 2, 4-dichlorophenoxyacetic acid. Plant Physiol. 27: 293-301.

———, J. H. REINHART, R. A. SCHERFF, AND L. C. SCHISLER. 1954. Metabolism of 2, 4-dichlorophenoxyacetic acid. III. Metabolism and per-

sistence in dormant plant tissue. Plant Physiol. 29: 303-304.

WOOD, J. W., J. W. MITCHELL, AND G. W. IRVING. 1947. Translocation of a radioactive plant growth regulator in bean and barley plants. Sci. 105: 337-339.

Tropical Silviculture

By I. T. Haig, M. A. Huberman, U. Aung Din, et al. FAO Forestry and Forest Products Studies No. 13, Food and Agriculture Organization of the United Nations, Rome, Italy, 1958. Vol. I (English), 190 pp., $2.00; Vol. II, 415 pp., $4.00; and Vol. III, 101 pp., $1.00.

Review by Frank H. Wadsworth,
Tropical Forest Research Center, U. S. Forest Service
Rio Piedras, Puerto Rico.

Tropical Silviculture undertakes an extremely ambitious task, the summation and evaluation of a century of silvicultural experience on nearly half of the world's forest land. This objective is approached in two ways: a review and summary of literature on this subject by the authors named above (Volume I) and publication of general invitation papers on this topic prepared for the Fourth World Forestry Congress in 1954 (Volumes II and III).

Volume I is published in English, French, and Spanish. It defines the limits of tropical forests on the basis of the "character" or the "flora" and thus includes some areas in latitudes as high as 30° on both sides of the Equator, yet excludes "temperate climate" areas within this zone. Three broad formations of mixed forest are recognized: wet evergreen, moist deciduous, and dry deciduous. Three additional specialized formations are discussed: mangrove, bamboo, and coniferous forest. Each of the formations and its silviculture are described for three major tropical forest regions: Asia, Africa, and America.

Volume I is comprehensive and cites a good list of references. It brings out those characteristics of tropical forests which, at least in degree, distinguish tropical silviculture from that of the temperate zone: heterogeneity of composition, the complexity of the selection of species to favor, and the difficulties which have been encountered in the encouragement of the natural regeneration of desired species.

The need for a world-wide review of tropical silviculture is evident in the numerous different techniques described in Volume I, many of them defined geographically by political boundaries. Many opportunities for regional coordination are apparent. This is particularly true in the American Tropics where the least progress in silviculture is reported. Suggestions for forest research are very much to the point, and may prove helpful in the many countries where forest investigation is new or is confined by very limited resources.

The temperate zone forester will find in Volume I a valuable general reference on tropical forestry. An enormous amount of information has been digested and presented in a readable and interesting manner. This is the only comprehensive reference now available in this field. It probably will not be surpassed for years to come.

Volumes II and III contain 15 papers on rain forest silviculture, 9 on teak, and others on pine, mangrove, bamboo, and other species. The sources of these papers are disperse, with 19 from Asia, 6 from the Pacific islands from the Philippines to Australia, 14 from Africa, and 5 from America. Printed in the language of the author, 32 papers are in English and 12 are in French, one of which has an English summary. Valuable as these papers all are, they do not form a coherent series with Volume I, which was based upon a much broader survey of literature. However, they do interest the same audience and are presumably more readily available in this form than as a part of the official proceedings of the Congress. As current statements of silvicultural techniques, they will prove valuable references to every tropical forester concerned with silviculture.

Photoperiodic Control of Bud Development in Coulter Pine and Bigcone Douglas-Fir

BY
HENRY HELLMERS

PHOTOPERIOD MAY BE an important factor in protecting Coulter pine (*Pinus coulteri* (D. Don)) and bigcone Douglas-fir (*Pseudotsuga macrocarpa* (Vasey) Mayr) against winter frost injury. Even though the trees in the field are subjected to extended warm periods that frequently occur during the mild winter months, the buds are prevented from bursting until March or April by the length of the photoperiod.

Both of these tree species have a center of distribution in the San Gabriel-San Bernardino Mountains of California. Here the pine has an elevational range of 5,000 to 6,000 ft. The fir range is approximately 2,000 to 5,000 ft. elevation. The pine extends north to Mt. Diablo in the San Joaquin Valley and south to Mt. San Pedro Martir in Lower California. The fir extends northward along the Coastal and Sierra Nevada Mountain Ranges to a line running approximately due east from Santa Barbara and has the same southern limit as the pine (Sudworth, 1908).

At the 2,400-foot elevation in the San Gabriel Mountains the temperatures during winter warm spells frequently exceed 80°F. The 22-year average mean for November through April is 49.7±3.7°F. Highs of over 80°F. and lows of below freezing have occurred in each of these six months. In some years one of the winter months may be warmer than April, May, or June (Sinclair, Hamilton, and Waite. 1953),

In such a climatic situation, apparently some other environmental factor than temperature controls the breaking of bud dormancy. One such factor could be photoperiod, which in the San Gabriel and San Bernardino Mountains varies from about 9 to 15 hours during the course of a year.

From the results obtained by many workers it is well known that photoperiod does affect the breaking of dormancy and growth of many tree species.[1] Dormancy of bigcone Douglas-fir and Coulter pine could not be broken by the application of gibberellic acid by Lockhart and Bonner

[1]Wareing 1956, Vaartaja 1957, Downs and Borthwick 1956, Wassink and Wiersma 1955, Pauley and Perry 1954, Gustafson 1938, and Kramer 1936.

The author is a plant physiologist at the California Forest and Range Experiment Station, which is maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California at Berkeley. The research described here was carried on cooperatively with the California Institute of Technology, using its facilities at Pasadena. Thanks are due to James A. Lockhart and James Bonner for their cooperation in this experiment; also, to Ralph Van Wagner of the Los Angeles County Foresters and Fire Wardens Department for providing bigcone Douglas-fir seedlings, and to Gustaf Juhren of the Angeles National Forest for Coulter pine seedlings.

(1957). Using Douglas-fir (*Pseudotsuga menziezii* (Mirb.) Franco) plants collected along an east-west transect of Oregon from an elevation of 4,000 ft. to 60 ft. Irgens-Moller (1957) studied the effect of temperature and photoperiod on bud bursting. The high-altitude plants showed a definite photoperiodic response, whereas the trees from the lower, warmer sites failed to show a significant response. Morris, Silen, and Irgens-Moller (1957), also working with Douglas-fir, suggested that there is a strong genetic control of bud-breaking time. In a study of Oregon and Washington strains of Douglas-fir planted under a series of environmental conditions, they observed that the relative time and order of bud opening among individual strains planted at different latitudes and altitudes remained fairly constant for successive years. The present study indicates that photoperiod controls bud dormancy and growth of 2-year-old seedlings of both bigcone Douglas-fir and Coulter pine.

Materials and Methods

Two-year-old seedlings of Coulter pine and bigcone Douglas-fir grown from seed collected in the San Gabriel Mountains were placed under two photoperiods. The two photoperiods used were one of 9 hours (short day) and one of 20 hours (long day). The short day was obtained in the greenhouse by enclosing a bench with light-tight curtains that were opened daily at 0730 and drawn at 1630. For the long days, natural day length was supplemented with incandescent lights of 100 foot-candle intensity at crown height. Minimum temperature in the greenhouse was not permitted to drop below 64°F.

The test continued from October 1954 to July 1955. Fourteen Coulter pines and 4 bigcone Douglas-fir trees, potted stock grown under natural day length, were used. These were equally distributed between the photoperiodic conditions.

Data were later obtained from a much larger sample of trees under the same test conditions, as described under the next heading.

Results

Bud enlargement in the Coulter pine started almost immediately under the 20-hour photoperiod. It started in both pine and fir within 6 weeks after having been transferred to long-day conditions. The time for completing the first flush of growth and the setting of buds varied for the species and for the trees within the species. In these phases of development the pine appeared to lag behind the fir by about a month. Coulter pine set new buds within 5 months of the start of the experiment (Fig. 1). Bigcone Douglas-fir had set new buds by the first of February, or only 4 months from the start of the experiment. Second flushes of growth were produced by plants of both species. The bigcone Douglas-fir trees produced a third growth flush before the first experiment was terminated in July (Fig. 2).

Buds on trees under the short photoperiod did enlarge during the course of the experiment. Buds on 3 Coulter pine trees expanded sufficiently in 6 months to produce a few needles. In general, the buds doubled in length over the 9-month period (Fig. 1). Bigcone Douglas-fir trees under the short day failed to develop new needles in the 9 months of the study; the buds enlarged only slightly.

These results were then confirmed on a larger scale. A new experiment was conducted from November 1956 to April 1957. About fifty 2-year-old seedlings of each species were distributed between long (20-hour) and short (9-hour) photoperiods. Six to 8 weeks after the start of the study the 22 Coulter pines and the 25 bigcone Douglas-fir seedlings under the long days had broken dormancy. At this time the 28 pines and the 26 firs under the short period were all dormant. After 4½ months, 8 pines and 5 Douglas-firs under

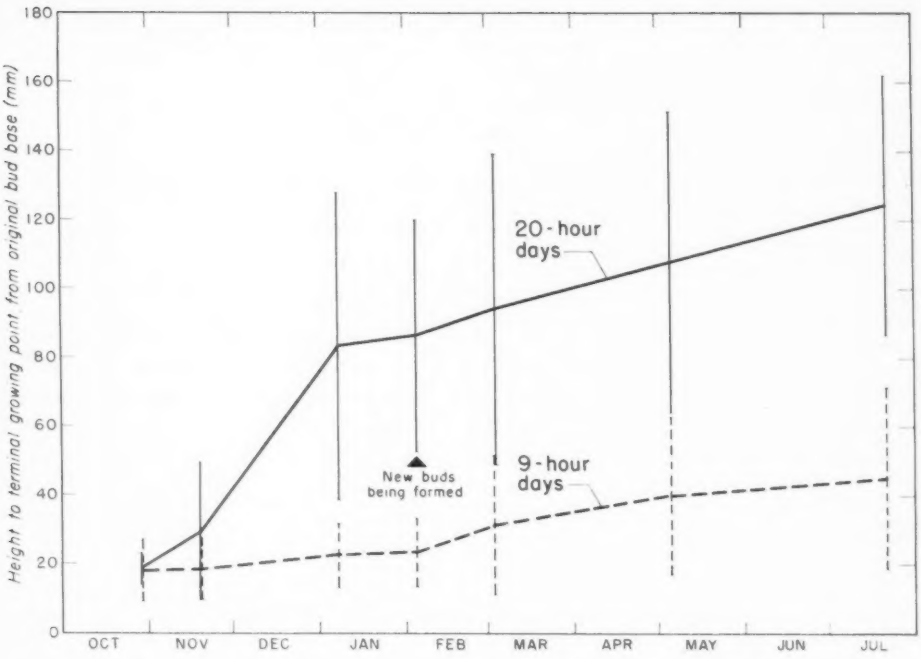

FIGURE 1. *Height growth of 2-year-old Coulter pine seedlings grown under 20-hour and 9-hour photoperiods for 9 months (October 1954 to July 1955). The vertical bars represent the standard deviation from the mean for 7 trees. For the 20-hour photoperiod the decrease in variability on February 4 reflects bud formation at the completion of the first flush of growth. The rapid increase followed by a decrease of variability after February 4 is caused by a second flush of growth.*

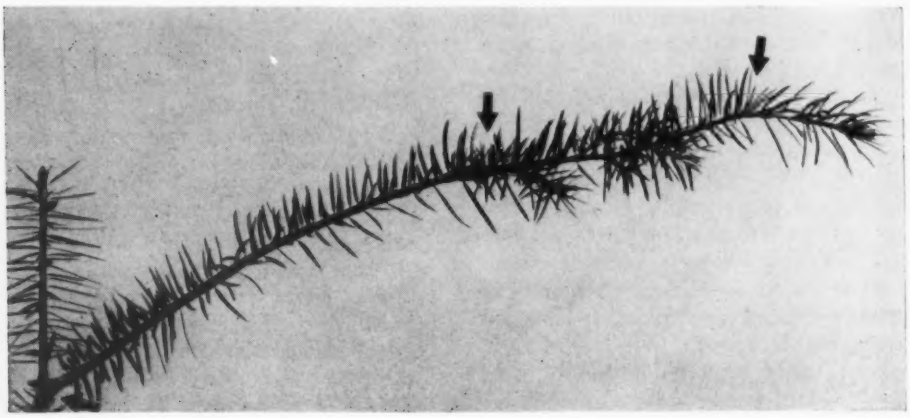

FIGURE 2. *Branch of bigcone Douglas-fir showing three flushes of growth produced between October 1954 and July 1955. Arrows indicate nodal points.*

the short photoperiod had broken dormancy. This timing corresponds with some of the earliest bud opening observed in the field. The 8 Coulter pines had developed some new needles along the bud axis, but the terminal bud itself did not open. The fir buds that opened failed to develop and died.

Discussion

A long photoperiod appears to promote bud opening in Coulter pine and bigcone Douglas-fir. However, a short photoperiod does not completely inhibit bud opening. The slowness and the low incidence of bud opening under short days and favorable temperatures in the laboratory explain why new, tender shoots are not formed in the field until the days lengthen and the danger of a winter frost is usually past.

The slow rate of bud bursting in the pine may be related to its natural distribution, which is both farther to the north and at a higher elevation than that of bigcone Douglas-fir. An experiment is being conducted to determine the photoperiodic threshold of both species.

Summary and Conclusions

Dormant Coulter pines and bigcone Douglas-fir seedlings were divided between a long (20-hour) and a short (9-hour) photoperiod treatment in a greenhouse during the winter months. Minimum temperatures were kept at 64°F. or above.

All plants of both species broke dormancy under the long photoperiod within 6 to 8 weeks. All plants under short photoperiod failed to break dormancy within this time.

The photoperiodic control over bud dormancy prevents the premature formation of new shoots during mild winters or unseasonable warm spells.

Literature Cited

Downs, R. J., and H. A. Borthwick. 1956. Effects of photoperiod on growth of trees. Bot. Gaz. 117:310-326.

Gustafson, F. G. 1938. Influence of the length of day on the dormancy of tree seedlings. Plant Physiol. 13:655-658.

Irgens-Moller, Helge. 1957. Ecotypic response to temperature and photoperiod in Douglas-fir. For. Sci. 3:79-83.

Kramer, Paul J. 1936. Effect of variation in length of day on growth and dormancy of trees. Plant Physiol. 11: 127-137.

Lockhart, J. A., and J. Bonner. 1957. Effects of gibberellic acid on photoperiod-controlled growth of woody plants. Plant Physiol. 32:492-494.

Morris, W. G., R. R. Silen, and H. Irgens-Moller. 1957. Consistency of bud-bursting in Douglas-fir. J. For. 55: 208-210.

Pauley, S. S., and T. O. Perry. 1954. Ecotypic variation of the photoperiodic response in Populus. J. Arnold Arbor. 35:167-188.

Sinclair, J. D., E. L. Hamilton, and M. N. Waite. 1953. A guide to the San Dimas Experimental Forest. U. S. For. Serv., Calif. For. and Range Exp. Sta. Misc. Pap. No. 11. 19 pp. Revised 1956.

Sudworth, G. B. 1908. Forest trees of the Pacific slope. U. S. Dept. Agric. 441 pp.

Vaartaja, O. 1957. Photoperiodic responses in seedlings of northern tree species. Canad. J. Bot. 35:133-138.

Wareing, T. F. 1956. Photoperiodism in woody plants. Annual Review of Plant Physiology 7:191-214.

Wassink, E. C., and J. H. Wiersma. 1955. Daylength responses of some forest trees. Acta Bot. Neerlandica 4: 657-670.

Influence of Potassium Fertilizer Level On Red Pine Planted at Various Spacings On a Potassium-Deficient Site

BY
S. O. HEIBERG
L. LEYTON
H. LOEWENSTEIN

A STRONG RESPONSE of planted trees to fertilization with logging slash, forest humus and potassium salts on a level sandy outwash plain on the Pack Demonstration Forest, located in the southeastern foothills of the Adirondack Mountains of New York, has already been reported (Heiberg and White, 1951). The site had been tilled for agriculture and probably grazed during the early part of the present century. The species planted included red pine (*Pinus resinosa* Ait.), eastern white pine (*Pinus strobus* L.), white spruce (*Picea glauca* (Moench) Voss), and Norway spruce (*Picea abies* (L.) Karst.).

The present article deals with several outgrowths of this original experiment, namely, the long-term effects on tree growth of varying amounts of potassium fertilizer and a preliminary estimate of the uptake and distribution in the tree of potassium, nitrogen, and phosphorus.

In order to evaluate the effects of differing amounts of potassium fertilizer, four 1/10-acre plots were established and fertilized in May, 1948. (Briefly mentioned in Heiberg and White, 1951.) These plots were located adjacent to the plots fertilized in 1943 and 1946 with the KCl and the K_2SO_4. The plantation, consisting of red pine, was established in 1930 at spacings varying from 4 × 4 feet to 6 × 6 feet. In common with other plantations on this plain, a retardation of growth occurred five to seven years after planting. This was still evident at the time of fertilizer application.

A more detailed description of the planting site has been presented previously (Heiberg and White, 1951). Several deep soil pits have been excavated on the 120-acre plain. The profile of the Hinckley soil revealed in a pit 165 feet from the experimental plots is described below:

Vegetation	Red pine plantation, with a few remnants of *Cladina* spp.
L	¾ inch of loose red pine needles, scattered twigs and bark scales.
F	¼ inch of loose material.

The authors are respectively Professor of Silviculture, State University College of Forestry at Syracuse University, Syracuse, N. Y.; University Lecturer and Demonstrator, Department of Forestry, Oxford University, Oxford, England; and Assistant Professor of Forestry, College of Forestry, University of Idaho, Moscow, Idaho.

Contribution from the Department of Silviculture, State University College of Forestry at Syracuse University, Syracuse, New York. This work was in part supported by the Charles Lathrop Pack Foundation Fund.

H Barely visible layer of black material. Very few fine feeding roots observed in F and H layers.

A_p 6 inches of 7.5 YR 4/2 to 4/4[1] thoroughly mixed humus and coarse sand. Several roots in this horizon, but it appears as if there is greater root distribution in the B horizon. Charcoal observed, but no remnants of the original A_2 horizon. pH 5.0.

B 7 to 9 inches of 7.5 YR 5/6 to 6/6 coarse sand. Numerous roots throughout horizon. pH 5.5. Blending into—

C 80 inches of 10 YR 5/2 to 7/2 fairly even textured, though stratified, coarse sand, pH 6.0. A few live roots observed in upper part of horizon. Strong concentration of new roots observed in brownish old root channels (probably from original forest). One such concentration begins 52 inches from surface and ends 82 inches from surface. This has resulted in coloring reddish-brown a six-inch vertical band through this coarse sand. No clear evidence that this root concentration enters the fine layer below. Sharp line of 10 YR 7/2 fine sand to 55 inches plus, pH 6.1. Several brown spots surrounding old dead roots. No live roots observed in this layer. Use of soil auger at bottom of pit did not result in passing through this fine sand. It appears that the deeper live roots noted in this profile all follow old root channels.

Experimental Procedure

The four 1/10-acre plots situated adjacent to each other in an L shaped pattern were treated with commercial potassium muriate (0-0-60)[2] as follows: Plot S, 50 lbs. per acre; Plot T, 100 lbs. per acre; Plot R, 200 lbs. per acre; Plot Q, 300 lbs. per acre. Thus on an acre basis, approximately 25 lbs. of elemental potassium were added to Plot S, 50 lbs. to Plot T, 100 lbs. to Plot R, and 150 lbs. to Plot Q. The appropriate amount of fertilizer for each row within each plot was weighed out and care taken to distribute it as evenly as possible on May 10, 1948. This was a few days after the soil had thawed from the winter frost and one to two weeks before elongation of the 1948 annual shoots. Plot K, located 66 feet to the north, was employed as a check.

The plots were measured during May 1957. A temporary 1/20-acre measurement plot was laid out in the center of each 1/10-acre experimental plot. The d.b.h. of each living tree was recorded, including those with double and triple stems. Height of the 30 tallest unforked trees was measured, utilizing a specially constructed wooden extension hypsometer. The height both at the end of the 1956 growing season and at the time of fertilization was determined.

Six trees, selected close to the mean diameter of the stand, were cut flush with the ground. The diameter of each four-foot section, as well as the tip section, of these sample trees was tabulated for form factor determination. Each tree, including stem, bark, branches, and needles, was then weighed on a spring balance. The d.b.h. of each was also recorded. This process was repeated for ten other experimental plots in the compartment. In this way the weight and the d.b.h. of a large number of individual trees were obtained. These data were later graphed and the green weight of the tree of mean basal area on each plot was estimated from the curve (Fig. 1).

An additional tree from each plot selected near the d.b.h. of mean b.a. was taken for the laboratory analyses. Each of these (Plot S excepted) was divided roughly into branches with foliage, older branches, and main stem and each compo-

---

[1]Color notations refer to Munsell soil color charts.

[2]Potassium muriate is commercial potassium chloride. In 100 lbs. of 0-0-60 there are approximately 60 lbs. of K_2O, or 50 lbs. of elemental potassium. One hundred lbs. per acre is approximately equal to 112 Kg per hectare.

nent weighed immediately as fresh material on the spring balance. The bark was later removed from the main stem and the wood and bark treated separately. On transport to the laboratory (within a few days of felling), a further subdivision of the foliage-bearing branches was made into recent (1956) needles and branches, and older (1954, 1955) needles and branches. The various components were dried in a kiln at 100° C for three days and then weighed as dry matter.

The stems were first reduced to chips in a mill, and, after thorough mixing, three representative samples were ground in a Wiley mill; the remaining components, bark, branches, and foliage, were ground directly in the Wiley mill and two to three samples of each taken for analysis.

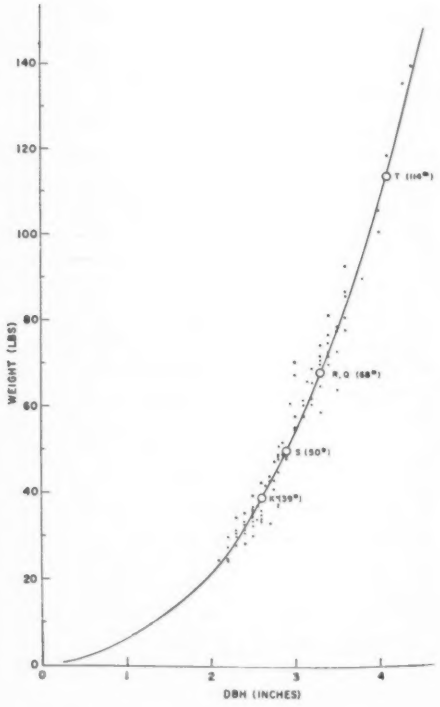

FIGURE 1. *Graph of green weight over d.b.h. for trees selected from 15 experimental fertilizer plots on compartment 26, Pack Forest. The weights of the trees of mean basal area on plots K, S, T, R, and Q are indicated on the curve.*

During October 1957 samples of current-year needles were obtained from three trees on each plot. The foliage was collected from the third whorl from the top on the north side of each tree. The needles were stripped from the branch in the field, subsequently dried in a circulating air oven at 70° C for 24 hours, ground in a Wiley mill and analyzed for potassium.

Soil samples were taken with a Cenco sampling tube. After removing the L and F layers, 15 cores from the six-inch surface mineral horizon of each plot were collected, dried, mixed, and passed through a 1 mm sieve.

Nitrogen in tissue was determined by the Kjeldahl method, phosphorus by the vanadate method employing a Bausch and Lomb colorimeter, and potassium by ashing the tissue, taking up in 4*N* HNO_3 and analyzing spectrometrically on a Beckman flame spectrophotometer.

The soils were analyzed for pH using the Beckman glass electrode on a soil paste, and for potassium by extraction with 1*N* ammonium acetate followed by spectrophotometric observations.

Growth Response

Stand and growth data are presented graphically in Figure 2. Average height refers to the mean height of the ten tallest trees on the plot, selected from the 30 trees measured. Volume figures represent total stem volume above ground, including bark. These were determined as a product of basal area, mean form factor, and the height of the tree of mean basal area, taken from a height-diameter curve. Total dry weight above ground was found by converting the green weight of the tree of mean basal area to dry weight, and multiplying by the number of stems per acre.

Number of stems per acre is about the same as the initial survival after planting

because in red pine—in contrast to white pine and white spruce—no subsequent mortality takes place due to this soil deficiency.

The fewer stems on Plot T is reflected in lower basal area, larger d.b.h. and greater height and height growth than that found on the other plots. But in production, both with respect to stem volume and dry matter, Plot T, which received 50 lbs. of potassium per acre, falls between Plot S, treated with 25 lbs. potassium per acre, and Plot R, which was treated with 100 lbs. of potassium per acre. On such a marginal site and for a species such as red pine that expresses only slight dominance, height growth of the tallest trees

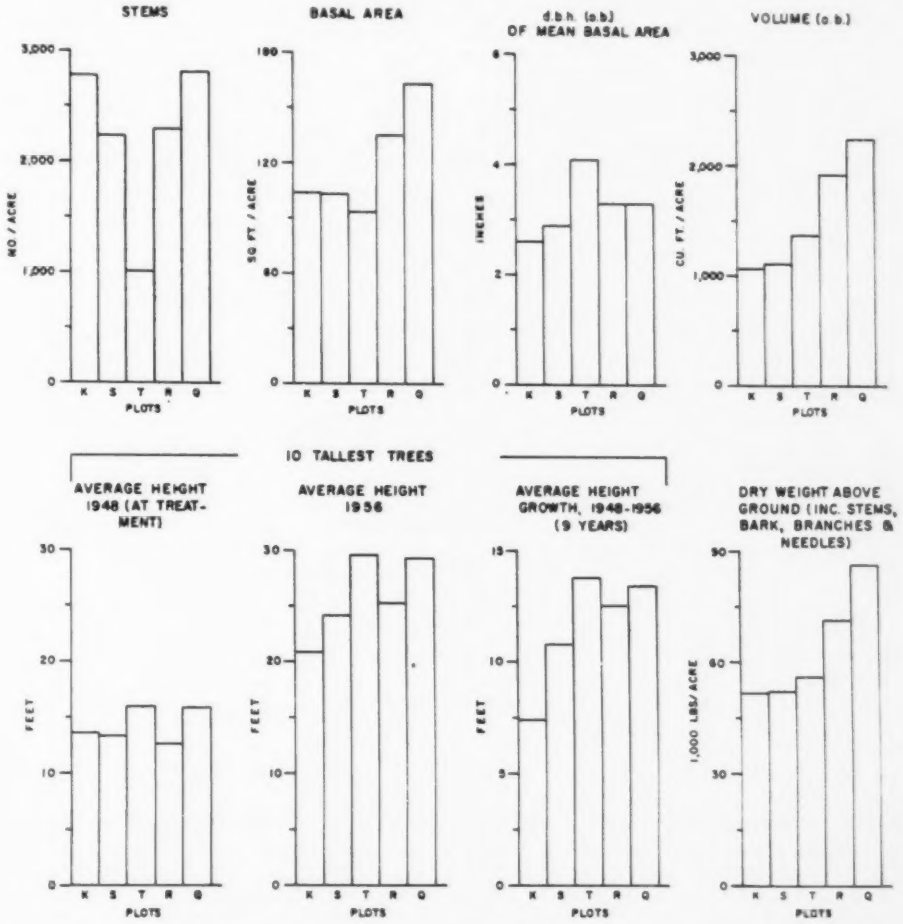

FIGURE 2. *Mensurational and weight data for plots fertilized with potassium at the following rates: K, control; S, 25 lbs./acre; T, 50 lbs. per acre; R, 100 lbs. per acre; Q, 150 lbs. per acre. Plot T was planted at a spacing of approximately 6 ft. × 6 ft., the others, approximately 4 ft. × 4 ft.*

appears to have limited significance as an indicator of true site quality.

The limited significance of height growth of red pine under these circumstances in indicating true site quality is illustrated by the data from a red pine spacing experiment established on the same outwash plain as the fertilization plots, separated by about 400 feet. Four ½-acre plots were planted with 2-1 red pine in 1929.

The spacings employed were 3 ft. × 3 ft., 4 ft. × 4 ft., 6 ft. × 6 ft., and 8 ft. × 8 ft.

The 4 ft. × 4 ft. plot was measured in May, 1957, the other three in May, 1958 (Table 1). It will be noted that on these plots also, height of the ten tallest trees, as well as height of the tree of mean basal area is greatly influenced by the spacing. Basal area is the same for the 3 ft. × 3 ft., the 4 ft. × 4 ft., and the 6 ft. × 6 ft. plots, indicating that this is the maximum basal area possible for this species, age, and site. The 8 ft. × 8 ft. plot has not yet reached this figure, but probably will in time. Total stem volume increases with spacing up through 6 × 6 feet, but the plot with 8-foot spacing, producing a larger proportion of branchwood than the others, lags behind. Six trees per plot, selected around the diameter of the stem of mean basal area, were weighed in order to determine production of dry matter. It was found that for the three plots with the widest spacings, the dry matter produced above ground, including needles, branches, bark, and stems, was practically the same. The lower production on the 3 ft. × 3 ft. plot may be due to the early intensive competition and hence possible inability of the roots to take advantage of a larger soil volume. This experiment illustrates an exception to the general rule that height growth of the leading trees in a forest stand is independent of distance between the trees. The explanation must be found in the extremely poor site and the low natural capacity of red pine to express dominance. The extremely low mortality of red

TABLE 1. Mensurational and weight data for spacing experiment (unfertilized plots).

Item	Plot spacing			
	3 ft. × 3 ft.	4 ft. × 4 ft.	6 ft. × 6 ft.	8 ft. × 8 ft.
Area measured[1] (acre)	.05	.05	.10	.10
No. trees per acre	4,800	2,600	1,400	740
Basal area per acre (sq. ft.)	103.8	103.5	103.2	86.2
D.b.h. of tree of mean b.a. (inches)	2.0	2.7	3.7	4.6
Ht. of tree of mean b.a. (ft.)	14.6	17.9	26.4	26.7
Ave. ht. of 10 tallest trees on plot (ft.)	20.7	22.9	32.0	30.4
Form factor	.698	.615	.543	.533
Volume (cu. ft) per acre	1,057.0	1,142.0	1,476.0	1,224.0
Green wt. of tree of mean b.a.[2] (lbs.)	18.5	44.5	80.7	149.0
Dry wt. of tree of mean b.a. (lbs.)	8.4	20.2	36.6	67.5
Dry wt. per acre (lbs.)	40,320.0	52,520.0	51,240.0	49,950.0
Stem wt.[3] (total in percent)	70.6	73.4	70.9	62.3

[1]The 4 ft. × 4 ft. plot was measured in May 1957, the others in May 1958.

[2]Six trees measured per plot. Weights of individual sample trees are as follows:
3 ×3 plot, 17, 17, 18, 18, 19, and 22 pounds; 4× 4 plot, 46, 45, 39, 52, 38, and 54 pounds;
6 × 6 plot, 78, 85, 82, 61, 76, and 77 pounds; 8 × 8 plot, 167, 133, 141, 155, 154, and 44 pounds.

[3]One tree measured per plot.

TABLE 2. Levels of soil and foliar potassium one and nine years after fertilization with potassium.

Plot	Treatment	Soil exchangeable potassium (ppm) 1949	1957	Potassium in current season needles (percent) 1949	1957
K	Control	16.4	11.0	0.33	0.31
S	25 lbs./A potassium	14.5	13.0	0.56	0.46
T	50 lbs./A potassium	16.6	13.0	0.58	0.45
R	100 lbs./A potassium	19.7	13.0	0.72	0.48
Q	150 lbs./A potassium	26.1	14.0	0.74	0.61

pine on this site is probably influenced by the latter.

Nutrient Relations

The levels of soil and foliar potassium, both for 1949, the year after fertilizer treatment, and for 1957, nine years after treatment, are shown in Table 2. In 1949, soil exchangeable potassium ranged from 16.4 ppm in the check plot (K) to 26.1 ppm in Plot Q, which received an application of fertilizer equivalent to 150 pounds of potassium per acre. The amount of exchangeable potassium varied but little from plot to plot in 1957, ranging from 11.0 ppm in Plot K to 14.0 ppm in Plot Q. On the other hand, after nine years, the concentration of foliar potassium still strongly reflects the fertilizer treatments. The 1957 needles from trees on the check plot (K) contained 0.31 percent potassium, a level considered deficient for red pine (Heiberg and White, 1951). On fertilized plots, the concentrations ranged from 0.45 percent for trees on Plot T to 0.61 percent on Plot Q. These figures from the fertilized plots represent a decline from the 1949 levels, but are still considerably higher than that for the check plot. In a nearby unfertilized plantation, vigorous, healthy trees analyzed in 1949 contained 0.50 percent potassium (Heiberg and White, 1951). This concentration is associated with good growth but may not be sufficient for maximum production.

The decline in soil exchangeable potassium, and to a lesser extent, foliar potassium, can probably be traced to two factors. Undoubtedly, over the nine-year span some leaching of potassium has occurred in this sandy soil. Then too, as the trees grow older more potassium is being immobilized in organs other than foliage. Possibly also, with an increase in crown volume demand for potassium is increased. This contention will be explored more fully below.

One of the aims of this investigation was to obtain some idea of the total nutrient demands of the trees. Owing to the magnitude of the work, involving the analysis of whole trees, it was not possible at this stage to sample more than one tree from each of four plots, the control and the plots receiving the three highest applications of fertilizer (50, 100, and 150 pounds per acre of potassium). Mensurational and weight data (on both a fresh and dry weight basis) for the four selected trees are given in Table 3. Data on nutrient content, in terms of both percent and absolute amount, are presented in Table 4.

The trees selected for analysis are all slightly larger than the true mean trees, the d.b.h.'s of which are shown in Figure 2. Therefore, in calculating the total uptake of nutrients (Fig. 3) and dry matter production on an acre basis the following procedure was employed. In each instance the figure obtained for the sample tree was recalculated for the tree of mean weight on

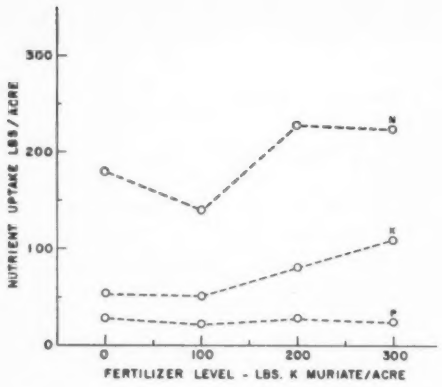

FIGURE 3. *Influence of levels of potassium fertilizer on total nitrogen, phosphorus, and potassium in above-ground portions of trees.*

the plot, determined from the weight-d.b.h. curve shown in Figure 1. This new value was then multiplied by the number of trees per acre for each particular treatment (Fig. 2).

It may be noted that the sample tree chosen from Plot T reflects the wider spacing in its larger d.b.h. and its greater height. Also, the dry weight data appear to indicate that the needles of trees planted at a spacing of 6 × 6 feet are retained longer than those of trees planted closer together.

In general the foliage and other organs of the control tree contain higher percentage contents of nitrogen and phosphorus than do those of fertilized trees (Table 4); this is what might be expected if the potassium fertilization stimulated dry weight production more rapidly than the uptake of these nutrients (false antagonism). In contrast to Plot T, the widely spaced plot, the increased growth on Plots R and Q has resulted in an increase in total uptake of nitrogen. The total uptake of phosphorus, however, appears to be more or less independent of the fertilizer influence (Fig. 3). This latter finding might be taken as suggesting a more than adequate phosphorus supply in the soil with luxury consumption taking place to a level determined more by the ready availability of the phosphorus than by actual requirements for growth.

TABLE 3. Mensurational and weight data for sample trees on different plots.

	Sample tree[1] on—							
	Plot K		Plot T		Plot R		Plot Q	
	(lbs.)	(percent of total)	(lbs.)	(percent of total)	(lbs.)	(percent of total)	(lbs.)	(percent of total)
				Fresh weights				
Needles and live branches	10.2	23.3	26.2	19.3	17.2	23.5	13.2	15.4
Dead branches	8.1	18.5	21.6	15.9	11.1	15.2	7.6	8.9
Stem wood and bark	25.5	58.2	87.8	64.8	44.9	61.3	64.9	75.8
	43.8		135.6		73.2		85.7	
				Oven dry weights				
Needles, 1956	1.6	7.5	3.4	5.2	3.5	10.5	3.5	9.0
Branches, 1956	0.5	2.4	0.9	1.4	0.7	2.1	0.6	1.5
Needles, 1954/55	1.8	8.5	6.7	10.2	2.4	7.1	2.5	6.4
Branches, 1954/55	1.3	6.1	3.5	5.3	2.4	7.1	1.6	4.1
Older branches	5.1	24.1	12.3	18.8	6.8	20.3	5.1	13.1
Stem bark	1.9	9.0	6.2	9.5	3.5	10.5	3.7	9.5
Stem wood	9.0	42.4	32.5	49.6	14.2	42.4	21.8	56.4
	21.2		65.5		33.5		38.8	

[1]The d.b.h. and height of sample trees were as follows: Plot K–3.0 in. and 16.5 ft.; Plot T–4.3 in. and 27.8 ft.; Plot R–3.4 in. and 19.3 ft.; Plot Q–3.6 in. and 27.0 ft.

TABLE 4. Nitrogen, phosphorus and potassium contents of sample trees on different plots.

	Plot K		Plot T		Plot R		Plot Q	
	Dry Wt. (percent)	Content (g)	Dry Wt. (percent)	Content (g)	Dry Wt. (percent)	Content (g)	Dry Wt. (percent)	Content (g)
				Nitrogen				
Needles, 1956	1.08	8.0	0.95	14.8	1.01	15.9	0.89	14.2
Branches, 1956	0.84	1.8	0.40	1.7	0.58	1.9	0.54	1.5
Needles, 1954/55	1.03	8.3	0.86	27.3	0.86	9.3	0.83	9.5
Branches, 1954/55	0.39	2.3	0.26	4.1	0.33	3.6	0.27	2.0
Older branches	0.23	5.3	0.19	10.6	0.21	6.5	0.21	4.8
Stem bark	0.33	2.8	0.29	8.1	0.31	5.0	0.32	5.4
Stem wood	0.090	3.7	0.065	9.6	0.090	5.8	0.078	7.7
Total per tree		32.2		76.2		48.0		45.1
				Phosphorus				
Needles, 1956	0.19	1.41	0.15	2.34	0.12	1.88	0.10	1.59
Branches, 1956	0.097	0.21	0.062	0.26	0.084	0.27	0.083	0.22
Needles, 1954/55	0.13	1.05	0.12	3.82	0.15	1.62	0.12	1.38
Branches, 1954/55	0.083	0.50	0.068	1.08	0.036	0.39	0.042	0.31
Older branches	0.035	0.81	0.030	1.68	0.025	0.78	0.015	0.35
Stem bark	0.042	0.36	0.044	1.23	0.028	0.45	0.029	0.49
Stem wood	0.010	0.41	0.0065	0.96	0.0072	0.46	0.0069	0.68
Total per tree		4.75		11.37		5.85		5.02
				Potassium				
Needles, 1956	0.32	2.37	0.43	6.71	0.49	7.69	0.61[1]	9.70
Branches, 1956	0.40	0.88	0.38	1.60	0.39	1.25	0.42	1.13
Needles, 1954/55	0.26	2.11	0.25	7.95	0.28	3.02	0.34	3.91
Branches, 1954/55	0.18	1.08	0.17	2.70	0.20	2.18	0.22	1.63
Older branches	0.043	1.00	0.040	2.24	0.050	1.56	0.071	1.63
Stem bark	0.12	1.02	0.09	2.52	0.12	1.92	0.17	2.86
Stem wood	0.024	0.98	0.024	3.54	0.028	1.81	0.027	2.67
Total per tree		9.44		27.26		19.43		23.53

[1]The value for potassium content of 1956 needles of the sample tree on plot Q was 0.49 percent. Possibly due to an irregularity in the analytical procedure, this figure was in conflict with that found in analyzing 1956 foliage from several other trees on the plot (0.61 percent). Therefore, this latter figure has been substituted for 0.49 percent in calculations.

It appears that at the wider spacing there is no overall extra utilization of the soil nutrients, but rather, in the case of nitrogen and potassium, an efficiency of utilization somewhat below that at the closer spacings. In his work on the nutrient uptake by young jack pine (*Pinus banksiana* Lamb.) plantations at different spacings, Adams (1928) came to a similar conclusion. This is illustrated more clearly when the total amount of potassium taken up by the stands is compared. Unfortunately, no data are available for the potassium content of the root systems of the sample trees but from the information provided by Rennie (1955) it would appear reasonable to assume that they contain about 13 percent of the total present in the whole tree. Thus in Plots K, T, R, and Q, the total uptakes would be in the order of 60, 59, 94, and 130 pounds per acre of potassium, respectively (Table 5). Plot R has absorbed 34 pounds more and Plot Q, 70 pounds more of potassium than the control. The total uptake on Plot T, the plot with wide spacing, is about that of the control. Apart from their very approximate nature, these calculations do not take into account extra potassium contained in the litter of the fertilized plots and would

therefore tend to underestimate the efficiency of utilization.

Moreover, Figure 2 discloses that Plot Q, which received 150 pounds of potassium per acre, the highest dosage, has increased its stem volume by 112 percent. The volume on Plot Q is 15 percent greater, and the dry weight production 21 percent greater, than that found on Plot R, which was treated with 100 pounds of potassium per acre. There is, therefore, further evidence that increase in growth may be obtained with even heavier applications of fertilizer. The indication that the curve for potassium uptake is still rising even at the highest dosage of fertilizer (Fig. 3), together with the evidence of increased growth with increased amounts of potassium, support the idea that the optimum dosage for this soil and species has not yet been reached.

In Table 6, the relative distribution in the various organs of the three nutrients investigated is presented in terms of their percentage of the total content of the above-ground parts of the tree. As will be seen, the foliage generally accounts for over 50 percent of the total nutrient content (the youngest foliage usually rather more than the remaining combined foliage), the branches for about 20 to 30 percent, the bark about 10 percent, and the remaining 10 to 20 percent by the stemwood. Actual values tend to vary according to the nutrient concerned and to a certain extent with the level of fertilizer. Generally speaking, however, these figures imply that even at this relatively early stage in the growth of the plantation, the removal of stems with bark would alone mean a loss of 35 to 70 pounds of nitrogen, 5 to 6 pounds of phosphorus, and 10 to 25

TABLE 5. Uptake of potassium on an acre basis by trees fertilized with varying amounts of potassium.

Plot	Treatment	K in above ground parts of trees (lbs/acre)	K in entire trees (incl. roots)[1] (lbs/acre)	K absorbed in excess of control (lbs/acre)	Excess K absorbed K added in fertilizer (percent)
K	Control	53	60		
T	50 lbs./a potassium	51	59	(—1)	
R	100 lbs./a potassium	82	94	34	34
Q	150 lbs./a potassium	113	130	70	47

[1]Roots estimated to contain 13 percent of total potassium in tree.

TABLE 6. Relative distribution of N, P, and K in the various organs (as percent of total above-ground content).

Organ	Nitrogen				Phosphorus				Potassium			
	K	T	R	Q	K	T	R	Q	K	T	R	Q
Foliage, 1956	25	19	33	31	30	21	32	32	25	25	40	41
Foliage, 1954/55	26	36	19	21	22	33	28	27	22	29	15	17
Small branches[1]	13	8	12	8	15	12	11	11	21	16	18	12
Large branches[1]	16	14	14	11	17	15	13	7	11	8	8	7
Stem bark	9	10	10	12	7	11	8	10	11	9	10	12
Stem wood	11	13	12	17	9	8	8	13	10	13	9	11

[1]Small branches bearing foliage, remainder equals large branches.

pounds of potassium per acre, according to the fertilizer dosage. At a later stage this loss would be much greater. Heiberg and White (1951) state that the total potassium content of the soil is of the order of 1.5 to 1.8 percent, i.e., over a depth of seven inches, more than 30,000 pounds per acre of total K, a figure which would theoretically support a considerable number of rotations. However, most of this potassium is unavailable as primary soil minerals and to quote from the above article "the small amounts of available potassium released through weathering and normally conserved by the humus of the forest soil in exchangeable form, apparently have been severely depleted through long agricultural use." Without some form of amelioration or increased weathering, the harvesting of a forest crop on such a soil would aggravate the situation still further.

Another point to consider is that as the crop matures, an increasing proportion of the nutrients taken up by the stand will be locked up in the stem wood, bark, large branches and roots. The observation that over 50 percent of the nitrogen, phosphorus, and potassium is contained within the foliage of the tree and will thus ultimately be returned into circulation is somewhat misleading, in this case at least, since the foliage of these pines corresponds to about three years' needle production whereas only one year's needles would normally be expected to be returned each year as litter. Furthermore, since this would involve only the oldest needles which generally contain the lowest contents, at least of nitrogen, phosphorus, and potassium, less than one-third of the total foliage content is returned each year to the nutrient cycle. In other words, of the total nutrient content of the tree (including roots) probably less than 10 percent is returned in the annual litter fall.

It is too early to evaluate the development of a new humus layer and its influence upon the soil. At the present stage of these plantations strong L and F layers have been formed, but only traces of an H layer. It is, however, very evident that the formation of a humus layer has progressed further on the fertilized plots than on the unfertilized. The weathering action of this new humus layer may in time release sufficient potassium from the primary soil minerals to sustain the potassium economy of the stand. It must also be considered that as the plantations develop, especially when stimulated by potassium fertilization, the root systems of the trees expand and may reach a larger volume of soil. Thus new resources of nutrients and water may be exploited (Heiberg and White, 1956). It is therefore conceivable that the effect of any future artificial fertilization may be lessened or even eliminated.

Conclusions

The site used for this experiment is marginal, with potassium as a limiting factor for tree growth. Red pine, the only species discussed in this paper, has a low native ability to express dominance in pure even-aged stands. Top height as an indicator of productivity for a particular site is, therefore, in this situation, of limited validity. Wide spacing has led to the production of larger diameter and greater height of individual trees, but basal area remains constant and on an area basis there is no apparent extra yield. Further, stands planted at a wide spacing have a rather lower nutrient uptake on an area basis, because of lower density, compared with the trees at the closer spacing. The pronounced influence of the potassium fertilizer is still evident almost ten years after its application, and the nature of the response curve, together with that from the composition of the foliage and the total uptake would suggest that still further increases in yield may be obtained from higher rates of application. The continued influence of the fertilizer is reflected in a better potassium status of the foliage, corresponding to the level of fertilizer applied. No doubt this effect has been maintained through a higher

potassium content of the annual litter fall. However, with the relatively greater increase in the proportion of the more permanent organs compared with the foliage (which contributes to the litter), and the continued leaching out of potassium from the litter by rain water, this effect must fall off eventually unless by the development of a new humus layer and a deeper root system the stand is able to obtain potassium from latent sources.

Depending on the amount of fertilizer applied and the spacing, it appears that between 0 and 47 percent of the added potassium has found its way into the growing crop, the plot with the wider spacing showing no greater potassium uptake than the control on an acre basis. At the closer spacing, there appears to have been a good return from the fertilizer, considering how readily this nutrient is leached from the soil and the gain in yield obtained.

Although at the present stage of growth less than 50 percent of the nitrogen, phosphorus, and potassium in the tree can be considered as permanently immobilized in the various organs, it still represents an appreciable drain on the limited capacity of the site to maintain a sufficient supply. Even at this stage, the loss to the site through removal of only the stems with bark would not be insignificant, and this loss would be expected to increase with an increase in the age of the timber removed. It is perhaps relevant to point out that in the case of calcium, which is to some extent selectively accumulated in the stem (Rennie, 1955), the loss from the soil would be even greater. The solution to these problems on poor soils of this kind is self-evident. Not only is fertilization required to produce a satisfactory yield, but also to provide, after logging, a satisfactory substrate for future growth. From the viewpoint of management, the removal of the bark *in situ* is obviously to be encouraged, whereas burning of the slash should be held to a minimum.

It need hardly be emphasized that the validity of conclusions based on the analysis of a few individual trees, extrapolated in each case to cover the whole stand, must be qualified by the probable occurrence of an appreciable sampling error. Although the samples from each tree were obtained after thorough mixing of each of the representative components, and the reliability of this confirmed by replicated analyses, some error must be involved in the estimates for a whole tree. Furthermore, while care was taken to select sample trees close to the mean d.b.h., and the data were subsequently adjusted to the actual mean tree for each plot, this does not necessarily signify a mean value for either dry weight production or for nutrient uptake. Unfortunately, in the absence of replicates, no estimate of the probable error can be made. Nevertheless, the results obtained not only are generally consistent within themselves, but agree in principle with previous data, as for example, those summarized by Rennie (1955).

Summary

Mensurational analyses have been conducted and estimates of total yield and uptake of nitrogen, phosphorus, and potassium have been made on a 29-year-old red pine plantation growing on a potassium deficient soil treated with 50, 100, and 150 pounds per acre of potassium as potassium muriate when the trees were 20 years old. The trees receiving 50 pounds per acre of potassium had been planted at a spacing of approximately 6 × 6 feet, the others, approximately 4 × 4 feet. Data obtained from these plots and from an experiment in which unfertilized trees were planted at spacings of 3 × 3 ft., 4 × 4 ft., 6 × 6 ft., and 8 × 8 ft. indicate that with this species and on this marginal site, height of the tallest trees does not appear adequately to indicate productivity. However, volume and production of dry matter per acre increase with an increase in fertilizer application. The height growth and nutrient uptake of individual trees at the 6 × 6 ft. spacing,

receiving 50 pounds of potassium per acre, was substantially greater than that found for trees at a 4 × 4 ft. spacing receiving up to 150 pounds per acre of potassium. However, on an area basis the yield at the wider spacing fell into line according to dosage, while the relative nutrient uptake tended to be lower. In terms of pounds per acre, the total nutrient uptake by the above-ground portions of the stands was estimated to be 140 to 230 for nitrogen, 22 to 28 for phosphorus, and 51 to 113 for potassium according to the level of fertilizer applied. With an estimate being made for potassium in the tree roots, the data suggest that the potassium uptake on the most heavily fertilized plot was 70 pounds higher than the control. If this extra potassium was derived entirely from the fertilizer the efficiency of utilization would be almost 50 percent. There are indications that still higher rates of potassium applications would probably be effective on this site.

Generally the foliage accounted for over 50 percent of the total nitrogen, phosphorus, and potassium in the tree, but it was estimated that less than one-third of this is returned each year to the nutrient cycle. Twenty to 30 percent of the nutrients are contained in the branches, about 10 percent in the bark, and the remaining 10 to 20 percent in the stem wood. Even at this stage of the plantation, the removal of stem with bark entails a loss to the site on the order of 35 to 70 pounds of nitrogen, 5 to 6 pounds of phosphorus, and from 10 to 25 pounds of potassium per acre. Compared with the relatively low content of available potassium in this soil, its loss is appreciable and would be expected to increase as the stand matures.

Literature Cited

ADAMS, W. R., JR. 1928. Studies on the tolerance of New England forest trees, VIII: Effect of spacing in a jack pine plantation. Vt. Agric. Expt. Sta. Bull. No. 282.

HEIBERG, S. O., and D. P. WHITE. 1951. Potassium deficiency of reforested pine and spruce stands in Northern New York. Proc. Soil Sci. Soc. Amer. 15: 369-376.

———— and D. P. WHITE. 1956. A site evaluation concept. J. For. 54: 7-10.

RENNIE, P. J. 1955. The uptake of nutrients by mature forest growth. Plant and Soil 7: 49-95.

A System for Analysis of Forest Succession

BY
JOHN P. DECKER

WITHIN UNDISTURBED FORESTS on mesic sites, some trees replace others and become conspicuous parts of a climax forest. Reviews by Bourdeau and Laverick (1958), Billings (1957), Kramer (1956), Oosting (1956), and Kozlowski (1949), as well as many earlier ones, indicate that much effort has been devoted to searches for physiological explanations of succession. Because of the long span of time and the many edaphic, biotic, atmospheric and genetic variables involved; physiology of succession may appear very complex. This is especially true if succession is considered at the community level. Analysis is simplified by considering instead the life histories of individual plants.

For example, a hypothetical forest is composed of an even-aged overstory of a shade-intolerant species *A* and an understory of a shade-tolerant species *B*. When a tree of species *A* dies, a tree of species *B* grows up and replaces it in the overstory. This is the large and conspicuous change that defines forest succession, and it is accompanied by changes in physical and chemical characteristics of soil and in microfauna, microflora and microclimate. Yet these changes present no distinct physiological problem that is easily definable in terms of tree behavior. Both overstory and understory trees are simply completing normal life cycles: seed → seedling → mature tree.

The key to the change lies in failure of second generation *A* to appear in the understory. Reason for the failure can be found by examining the life history of second generation *A*. Abundant viable seed is cast by the *A* overstory. It germinates and many seedlings appear on the forest floor. They may survive several years, but sooner or later they are killed by summer drought. An *A* seedling is susceptible to drought because of an unfavorable proportion of leaves to roots that developed because of shading. Although upper needles receive enough light for maximum photosynthesis, lower ones are shaded further by upper ones and never reach maximum photosynthesis. A carbohydrate deficiency develops as a result. Root growth is more affected by this deficiency than is top growth, perhaps because roots are farther from the carbohydrate source. Seedlings of *B* are not so affected because all leaves receive enough light for maximum photosynthesis.

In this hypothetical sequence (essentially the classical Fabrician sequence), behavior of the shade-tolerant species was relatively uninteresting in that ordinary life cycles were completed with no definite problem to be explained. As indicated by the summary above, this type of succession can be explained most easily by analyzing the interrupted life history of the shade-intolerant species.

Because a life history can be resolved

The author is Plant Physiologist, U. S. Department of Agriculture, Arizona State College, Tempe, Arizona. However, this paper was written and submitted privately, and opinions expressed are not necessarily shared by any other member of the department. The author is grateful to V. M. Dolphin and E. L. Parker for several valuable suggestions.

into an incomprehensibly large number of events, some systematizing device is needed. A plane of events is one such device.

Analysis of a Life History as a Plane of Events

A life history can be described as a chain of events, with each event involving a whole plant. For example, a seed germinates, the seedling grows to be a sapling, the sapling is killed by fire. The same life history can be stated in terms of several chains of organ events, that is, in terms of what happens to leaves, roots and stem. Or it can be described in terms of a still larger number of tissue events. The resolving can be done repeatedly down through cellular events, subcellular events, colloidal events and molecular events. That is, if information were available, a life history could be described as an incomprehensibly large number of molecular events. Environmental factors can be resolved similarly; for example, illumination of a shoot, illumination of a mesophyll cell, a photon striking a chlorophyll molecule.

It is convenient to think of each size-order as symbolized by a horizontal chain of appropriate size, with each link representing an event. A graded series of such chains are stretched taut and parallel, one directly above another, as on a rectangular frame, so that they describe a plane. The horizontal axis of the plane represents time of an event, the vertical axis represents size of object involved in an event. Each bit of information about a life history fits at one place on the plane, with its position describable in term of rectangular coordinates.

Relationships among events of the several size-orders and time-orders can be represented as arrows symbolizing "leads to." Failure of an *A* seedling in a Fabrician sequence can be representd by diagonal arrows leading from certain organ events (behavior of lower needles) to certain system events (behavior of root system with respect to shoot) to certain plant events (desiccation and death of a seedling). Any given event can be resolved into the next suborder of component events (perpendicular to time) and it can be related to a preceding and a following event of the same size-order (parallel to time). Whether causality can be assigned to any relationship thus indicated depends primarily on the definition one chooses to assign to that nebulous abstraction *cause*.

Application of a Plane of Events

Systematizing the events comprising a life history clarifies relationships among events and thus provides a convenient means of examining the factual basis for inferences. For example:

1. During a graduate seminar a question arose as to why eastern white pine (*Pinus strobus* L.) does not occur on certain good hardwood sites in the Adirondack Mountains. A lively and speculative discussion developed that centered on comparative chemical properties of soils on these sites and on nearby sites where white pine grew well. Upon checking the problem and the discussion against a plane of events, the following became apparent: the discussion was based on an implicit assumption that the problem event (behavior of whole plants) was a consequence of certain prior molecular events (chemical properties of soil); none of the intervening events had been established or even identified; the unrecognized question of prime importance was at the whole plant level and concerned the stage at which the life cycle of white pine was interrupted on these sites; chemical properties of soil might have no important relationship to the interruption; the premature discussion at a molecular level was a kind of inferential broad-jump across the plane of events.

2. In another session of the same seminar a question arose as to why Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) does not occur on certain small land features interspersed in Douglas-fir forests of British Columbia. Field studies showed

large and consistent differences of pH between this soil and that immediately adjacent. Speculative discussion centered on how western redcedar (*Thuja plicata* Donn) was able to tolerate the low pH of these sites whereas Douglas-fir was not. Referring the discussion to a plane of events revealed the same defects as in the preceding example, with another complication added by an irrelevant question about a second species. That is, the discussion was based on an implicit assumption that the problem event (behavior of Douglas-fir trees) was causally related to certain prior ionic events (pH of soil); none of the intervening steps had been established or even identified; the unrecognized problem of prime importance was at the whole plant level and concerned the stage at which the life cycle of Douglas-fir was interrupted on these sites. The real problem is concerned only with the absence of Douglas-fir. The question about western redcedar is interesting in itself, but it has no necessary relationship to the Douglas-fir problem, and it only beclouds that problem.

3. In another session of the same seminar a debate developed on the perennial question of whether, in the Fabrician sequence, shading or drought is the more important. Referral to a plane of events revealed no disagreement as to events or simple relationships: because of shading, a seedling develops more shoot than root; because of this preponderance of shoot, the seedling is susceptible to drought. All disagreement was identified as arising from the vague term "important." Questions such as "Isn't drought *really* more important because it does the actual killing?" do not have consistent meanings. Their meanings depend on which of the many definitions one chooses to assign to "important." Seemingly, the only consistent denotation of "important" is "worthy of attention." The word is useful for arousing interest or getting attention, but it is nearly useless in analytical thinking.

4. Several professional ecologists have expressed opinions privately to me to the effect that searches for explanations of forest succession would be greatly expedited by further information about tree physiology. A related opinion was stated by Lutz (1957): "As experimental ecology develops more and more along physiological lines, silviculturists may look forward to the day when much of the present uncertainty will be removed from their important task of regeneration and culture of forest crops." Although these statements may be pleasing to a plant physiologist, they are not necessarily true. In explaining a successional sequence, the critical first question is autecological. It concerns that stage at which the life cycle of the succeeded plant was interrupted and the environmental factor most directly responsible for the interruption. A review of obvious sources shows that such information about most successional sequences in major forest types is lacking or at least not readily accessible. Internal mechanics of any given interruption can probably be explained readily on the basis of already familiar physiological principles, once the gross cause of a failure becomes known. Thus, the information most critically needed to begin the elucidation of most forest successional sequences is clearly autecological information. To assume that more physiological information would necessarily expedite matters is to jump inferentially from unspecified physiological events to unknown larger consequential events (behavior of whole plants and stands).

Summary

Systematic analysis of life histories of succeeded trees according to a plane of events facilitates explaining some forest succession.

Literature Cited

BILLINGS, W. D. 1957. Physiological ecology. Ann. Rev. Plant Physiol. 8: 375-392.

BOURDEAU, P. F. and M. L. LAVERICK. 1958. Tolerance and photosynthetic

adaptability to light intensity in white pine, red pine, hemlock and ailanthus seedlings. For. Sci. 4: 196-207.

KOZLOWSKI, T. T. 1949. Light and water in relation to growth and competition of Piedmont forest tree species. Ecol. Monogr. 19: 207-231.

KRAMER, P. J. 1956. The role of physiology in forestry. For. Chron. 32: 297-308.

LUTZ, H. J. 1957. Application of ecology in forest management. Ecology 38: 46-49.

OOSTING, H. J. 1956. The study of plant communities. W. H. Freeman and Co. San Francisco. 440 pages.

The Native Pinewoods of Scotland

By H. M. Steven and A. Carlisle, Oliver and Boyd. Tweeddale Court, Edinburgh. 368 pp. 1959. Price 63s ($8.82).

Review by Stephen H. Spurr
University of Michigan, Ann Arbor

Professor Steven of the Department of Forestry at the University of Aberdeen and Dr. Carlisle, whose 1954 doctorate thesis submitted to that University forms the basis for six chapters, have combined to summarize the history and present status of Scots pine in Scotland. Emphasized are the post-glacial history of the country, the impact of man on the forests from Mesolithic times to the present, a detailed description of the residual natural pinewoods, and a study of the morphological variation of the species in these woods.

Some 35 natural pinewoods of the original Caledonian Forest persist in Scotland in eight general areas. These range in size from a few acres to several thousand and occur in the Highlands from practically sea level to about 2000 feet. Most of the pines are old, the principal age class being from 140 to 190 years, with few surviving beyond 250 years. (Plantations date from at least 1600 so age alone is not proof of natural conditions). The trees are not large, being 60-70 feet high for the most part, with exceptional trees reaching 90 feet in height and 4 feet in diameter.

The interest in the study lies in the extensive knowledge of the past forests from pollen studies, the evidence of the impact on the forest of peoples after peoples who colonized or invaded Scotland, and the general picture presented of how a few acres of woodlands have persisted through the centuries to create a living legacy of the forests of the past.

Despite the long history of man in Scotland, it was apparently not until the middle ages that the native woodlands were greatly altered by logging, land-clearing, and other forms of destruction. As early as 1329 timber was imported from the Baltic lands, and in 1457 the first legislation to encourage planting was enacted. By 1503, it was stated that the woodlands of Scotland were destroyed, the burning of green wood from living trees was prohibited, and landowners were to plant at least one acre of woodland where there was no great woodland or forest. The Highland woodlands, however, survived because of the difficulty of transporting logs and lumber by land, and it was not until the end of the eighteenth century that logging here reached its peak. In the same century, large scale planting was initiated, and the question of the relative value of plantations and native stands was the subject of lively controversy.

The largest part of the book consists of detailed descriptions of the residual stands and the study of morphological variation in these stands. In this latter section, attention is given to crown forms, bark variants, leaf morphology and persistence, cone form, seed, and flower variations. These morphological variations were found to be essentially similar to those described for south Sweden.

While much of the book presents detailed information of primary interest to local foresters and forest owners, "The Native Pinewoods of Scotland" presents to all readers a clear picture of the historical development and present status of one of the more interesting forest areas in western Europe.

In Vitro Culture of Pine Roots and the Use of Pinus serotina *Roots in Metabolic Studies*

BY
ROBERT L. BARNES
AUBREY W. NAYLOR

APPLICATION of sterile culture techniques to the study of root physiology has provided a rapidly growing body of knowledge about synthetic capabilities, biochemical differentiation, and environmental factors affecting growth patterns of roots. Recent reviews by Street (1957) and White (1951) describe past progress in the culture of roots and effectively summarize the information gained through the use of sterile culture techniques. Although roots of a number of species have been successfully grown in culture, the specialized nutritional and environmental conditions required by many others remain to be elucidated before they can be grown continuously in culture.

Relatively few studies with isolated roots of trees have been reported. Isolated roots of three pine species, Scotch pine (*Pinus sylvestris*) (Slankis, 1947, 1948a, 1948b, 1949a, 1949b, 1950, 1951), Japanese red pine (*Pinus densiflora*) and Japanese black pine (*Pinus thunbergii*) (Isikawa, 1956) have been studied. Among woody angiosperms only blackwood acacia (*Acacia melanoxylon*) (Bonner, 1942) and black locust (*Robinia pseudoacacia*) (Seeliger, 1956) roots have been successfully cultured.

In the investigation reported here five species—pond pine (*Pinus serotina* Michx.), sand pine (*Pinus clausa* (Chapm.) Vasey), longleaf pine (*Pinus palustris* Mill.), slash pine (*Pinus elliottii* Englm.) and loblolly pine (*Pinus taeda* L.) — were successfully grown in sterile culture and maintained in subculture for more than two years. Pond pine roots have been subjected to more intensive experimentation than any others. The present paper describes experiments contributory to the development of satisfactory growing conditions for the roots *in vitro* and some preliminary metabolic studies done with the roots.

Materials and Methods

Seeds of pond pine used in this study were collected from two mature wind-pollinated trees in widely separated stands of the species in Florida. Because roots from seeds of the two trees grew at different rates and even the roots from seeds of the same tree were not uniform in growth rate, it

The authors are respectively Frasch Foundation Research Associate in Plant Physiology and Associate Professor of Plant Physiology, Department of Botany, Duke University, Durham, N. C.

The work reported here embodies a portion of a thesis submitted to the Department of Botany of Duke University by the senior author, in partial fulfillment of the requirements for the Ph.D. degree. During 1955-1957 the senior author held a National Science Foundation pre-doctoral fellowship. Major support for the work was provided by the Herman Frasch Foundation. Thanks are due to T. O. Perry, School of Forestry, University of Florida, for providing the seeds used in this study.

was necessary to produce a supply of clonal stock for discriminating tests. Although the results reported here are limited to one clone, the general findings are considered to be representative for the species as a whole.

Germ-free seeds were obtained by surface sterilization in a continuously agitated dilute solution of commercial Clorox (5.25 percent sodium hypochlorite). After thoroughly rinsing and soaking for 3 to 6 hours in sterile water, the seeds were germinated on sterile 1 percent agar in Petri dishes. Excised roots were grown in 125 ml Erlenmeyer flasks containing 50 ml of nutrient solution. After successful growth was obtained, all cultures were routinely kept in complete darkness at 23° C. Occasional brief observations were made in the light, and subcultures started from root sectors at intervals of approximately 30 days.

Several criteria of growth were used. Measurements were made on the length of the entire root system (main axis plus laterals) after subculturing and transferring to the test medium. Records were also made of the number of laterals produced and of the number of laterals living at the end of the test. Sometimes laterals initiated during a test died before the end of a test period; also, in some cases, lateral tips initially present on a subcultured segment died during an experiment. If more laterals died than were formed, negative numbers were recorded.

Where studies were being made on the ability of the roots to use certain substrates, the substance being tested was added to the standard medium and incubations carried out in 125 ml flasks for predetermined time intervals. Incubations with radioactive substrates were performed in minimal amounts of liquid. Usually 1 or 2 μc of radioactive material was in 200 μl of solution contained in a 2 ml Pyrex volumetric flask. Just enough liquid was present to bathe the volume of root tissue necessary for chromatographic analysis.

Tissues used in chromatography were washed free of adhering test solution, killed in boiling 80 percent ethanol, and further extracted with boiling water. The combined ethanol-water extracts were then reduced to approximately 0.1 ml by blowing a stream of dry, filtered air over the surface. An aliquot of the concentrate was chromatographed on sheets of 18¼ x 22½ inch Whatman No. 1 paper with water saturated phenol in the first direction and n-butanol-propionic acid-water in the other (Benson *et al.*, 1950). Where radioactive substrates were used, the chromatograms were exposed to No-Screen X-ray film for two weeks or more to locate the labeled compounds. The amount of radioactivity contained by each compound was determined by counting the area directly on the paper with a Geiger-Müller tube.

Amino acids, sugars, and organic acids were identified by their R_f values. In addition, specific spray reactions were used wherever feasible (Block *et al.*, 1958).

Results

Development of the Culture Medium

Initial attempts to grow the roots in continuous culture were made using White's (1943) and Slankis' (1950) media. Constant illumination of 500 footcandles (*ca*) intensity prevailed in the growing room. Finally, a test revealed that the roots grew much more rapidly and appeared more normal in branching habit when grown in the dark (Table 1). Subsequently, all cultures were kept in total darkness except when being briefly observed, measured, or subdivided and transferred.

Examination of Table 1 further shows that Slankis' medium was superior to White's for growing pond pine roots. After adjusting the sucrose concentrations of the two media to the same level (7 percent), and then testing each supplementary component of the two media on a comparative basis it was shown that glycine (3 ppm) was inhibitory. Absence of glycine, therefore, accounted for the superiority of

Slankis' medium.

Results of a test of the effect of glycine are given in Table 2. All concentrations of glycine used inhibited growth in length. When two dimensional chromatograms of water and alcohol soluble extracts of glycine-inhibited roots were obtained, however, it was obvious there was little free glycine within the roots, but there was a very large accumulation of serine. Substitution of serine for glycine in Slankis' medium resulted in an inhibition pattern (Ta-

TABLE 1. Growth of pond pine roots in light or in darkness in White's and Slankis' media during a 30-day period.[1]

Medium	Culture conditions	Growth in terms of—		
		Increase in number of all laterals	Increase in number of living laterals	Growth in length of all tips (mm)
White's,	Light	3.8	0.3	8.8
4 percent sucrose	Dark	5.0	2.0	18.2
Slankis',	Light	5.4	1.4	39.4
7 percent sucrose	Dark	18.6	13.5	110.4

[1]Based on 10 roots per treatment.

TABLE 2. Effect of glycine, serine, and glycine-arginine concentration on growth of pond pine roots in Slankis' medium for 30 days.[1]

Substance tested (ppm)		Growth as measured in—		
		Increase in number of all laterals	Increase in number of living laterals	Growth in length of all tips (mm)
Glycine				
0		8.0	6.4	120.4
0.1		8.8	6.8	90.8
1.0		12.6	10.8	92.8
10.0		1.0	—7.0	1.8
100.0		0.0	—5.6	1.6
Serine				
0		13.0	12.8	158.2
0.1		7.8	7.8	122.2
1.0		11.5	10.5	105.4
10.0		9.6	7.6	17.0
Glycine	Arginine			
0	0	5.0	2.6	80.8
3	0	4.4	—6.0	37.0
3	1	4.8	1.0	76.2
3	10	4.8	1.2	59.6
3	50	2.8	—1.8	24.2

[1]Based on 5 roots per treatment.

ble 2) almost identical with that previously obtained with glycine. Earlier work by Skinner and Street (1954) with excised groundsel roots indicated that arginine could possibly prevent glycine inhibition. When arginine was added to the pond pine root medium in the proper concentration, glycine inhibition did not occur (Table 2). One ppm of arginine prevented inhibition by 3 ppm glycine. The mechanism by which arginine antagonizes glycine inhibition has not been determined.

Preliminary tests of carbohydrate sources indicated that sucrose was superior to either glucose, fructose, or glucose-fructose mixtures in supporting growth of pond pine roots. A test was therefore run to determine the optimum concentration of sucrose for growth. Results of this test showed that 8 percent sucrose supported growth at the maximum rate.

Other tests showed that the concentration of minerals—including iron, which is often a critical factor (Street, 1957)—and the pH of Slankis' medium were satisfactory for the growth of pond pine roots. Pyridoxine and nicotinic acid (but not choline or thiamine, which were used by Slankis) appeared to be slightly stimulatory. As a result of these studies, the basic medium adopted for the remainder of the studies consisted of: mineral medium of Slankis (1950), 8 percent sucrose, 1.25 mg per liter $Fe_2(SO_4)_3$, 0.1 mg per liter pyridoxine, 0.5 mg per liter nicotinic acid, and 0.05 mg per liter thiamine-HCl.

Tests of Additives on Rate of Growth

Investigations on vitamin requirements. Preliminary tests indicated that thiamine additions were not necessary for satisfactory growth of isolated pond pine roots. In fact, concentrations greater than 1 ppm inhibited growth. Subsequent measurements, using a microbiological procedure, made it clear that pond pine roots can synthesize thiamine. Roots grown from 2-3 cm sectors when subcultured every two months had after 6 months in the absence of thiamine 4.48γ of the vitamin per gram fresh weight, whereas those cultured in the presence of thiamine had 14.28γ in a corresponding amount of tissue. The question then arose as to whether thiamine is required in normal metabolism. Evidence that thiamine actually is necessary was obtained by adding oxythiamine, an antagonist to thiamine (Woolley, 1952), to the medium. Oxythiamine was found to inhibit growth; and inhibitions caused by 10 ppm oxythiamine were completely reversed by 1.0 ppm thiamine (Table 3).

Many roots do not synthesize pyridoxine rapidly enough to support maximum growth. Addition of 0.1 ppm pyridoxine to Slankis' medium improved the growth of pond pine roots about 15 percent. When desoxypyridoxine, a pyridoxine antimetabolite, was substituted, growth was inhibited. Reversal of the inhibition caused by 10 ppm of desoxypyridoxine was obtained by the addition of 1 ppm pyridoxine (Table 3). These results supplied evidence that pyridoxine functions in the normal metabolism of the root.

A number of other vitamins including para-aminobenzoic acid, nicotinic acid, vitamin B_{12}, folic acid, ascorbic acid, riboflavin, and choline were tested as additives. None stimulated growth significantly; most were inhibitory when used at concentrations greater than 1.0 ppm. As yet analogues of these vitamins have not been tested.

Effects of auxins and other growth regulating compounds. In an effort to increase growth rates beyond those already obtained, the effects of indoleacetic acid (IAA), naphthalene-acetic acid (NAA), triiodobenzoic acid (TIBA), kinetin, and gibberellic acid were determined. All of these compounds inhibited growth when added at concentrations of 1.0 ppm. IAA (10^{-5} ppm) and TIBA (10^{-2} ppm) appeared to stimulate growth slightly (Table 4). IAA, NAA, and kinetin caused pronounced changes in the branching habit of pond

TABLE 3. Reversal of vitamin analogue inhibition of pond pine root growth by added thiamine and pyridoxine.

Substance tested (ppm)		Growth as measured in—		
		Increase in number of all laterals	Increase in number of living laterals	Growth in length of all tips (mm)
Oxythiamine[1]	Thiamine			
0	0	9.8	8.5	120.0
10	0	2.5	—3.2	48.1
10	1	6.8	4.4	138.8
10	10	15.4	13.5	84.3
10	100	5.5	4.4	30.6
Desoxypyridoxine[2]	Pyridoxine			
0	0	3.4	0.9	58.1
10	0	0.9	—1.9	35.0
10	1	3.3	1.6	70.0
10	10	0.6	—1.6	21.2
10	100	0.6	—4.8	20.6

[1]Length of test 40 days; 5 roots per treatment.
[2]Length of test 30 days; 8 roots per treatment.

TABLE 4. Stimulation of growth of pond pine roots by IAA and TIBA in a 30-day test.

Substance tested (ppm)	Growth as measured in—		
	Increase in number of all laterals	Increase in number of living laterals	Growth in length of all tips (mm)
Indoleacetic acid[1]			
0	16.4	15.6	123.2
10^{-3}	17.2	16.2	165.3
Triiodobenzoic acid[2]			
0	14.4	12.6	170.5
10^{-2}	14.8	12.5	231.0

[1]Twenty roots per treatment.
[2]Twelve roots per treatment.

pine roots. A concentration of IAA just high enough to inhibit growth in length stimulated the formation of numerous short, clustered, lateral roots. Comparable growth responses were obtained with 10^{-2} ppm NAA. But a different response was obtained with kinetin. At a concentration of 1 ppm numerous short, dichotomously branched laterals were formed, closely resembling mycorrhizae. A similar type of branching has been obtained with Scotch pine roots treated with IAA and NAA (Slankis, 1948b, 1949b, 1950, 1951). Though kinetin was one of the most effec-

tive inducers of dichotomous branching, similar branching followed the addition of many other apparently unrelated compounds including vitamins, antivitamins, and amino acids. Thiamine, pyridoxine, nicotinic acid, and folic acid when added at concentrations greater than 10 ppm, sulfanilamide at 1 to 10 ppm, and the amino acids glycine, serine, ornithine, citrulline, and arginine at concentrations greater than 1 ppm were all effective.

Effects of respiratory poisons on growth. Preliminary to biochemical studies on respiration, it was thought desirable to determine the overall effect of certain poisons on growth of isolated roots. Growth responses in the presence of the following respiratory inhibitors were determined: dinitrophenol (DNP), malonic acid, sodium fluoride, sodium arsenate, sodium arsenite, and iodoacetic acid.

When added to media buffered at pH 5.2, each of the poisons except malonic acid inhibited growth. Malonic acid is known to be most effective as an inhibitor, however, when used at a relatively low pH (about pH 3.0). Since pond pine roots did not grow satisfactorily when the pH of the medium was below 4.0, malonic acid could not be used at its optimum pH.

Practically complete inhibition of growth was obtained with the lowest concentration of DNP which was tested (10^{-6}M). The critical concentration of sodium fluoride was found to be between 10^{-3} and 10^{-4}M. Sodium arsenate inhibited growth about 80 percent at a concentration of 10^{-5}M; arsenite was extremely inhibitory at a concentration of 10^{-5}M; and iodoacetic acid inhibited growth about 50 percent when the concentration was 10^{-5}M.

Metabolic Studies

In addition to studying the effects of supplements of vitamins, amino acids, antimetabolites and respiratory poisons on the growth and form of isolated root cultures of pond pine, effects of the several treatments on presence and relative pool sizes of free amino acids, sugars and sometimes organic acids were determined. Such information was obtained chiefly by application of paper chromatographic, radio-tracer and radio-autographic techniques. Deviations from normal in pool size of important alcohol-water soluble metabolites were striking in many of the treatments employed.

Effects of respiratory inhibitors. Dinitrophenol, sodium arsenate, sodium arsenite, and iodoacetic acid altered the free amino acid pattern in pond pine roots markedly. In all cases, the levels of free amino acids declined drastically. The only conspicuous increase noted was in the amount of asparagine.

Studies with antimetabolites. As is shown in Table 3 the analogues of thiamine and pyridoxine markedly inhibited growth. In an attempt to learn which metabolic pathways were affected, paper chromatographic analyses were made. Chromatographed extracts of oxythiamine-inhibited roots revealed striking increases in the amounts of free alanine and serine. These amino acids would be expected to accumulate if oxythiamine were interfering with the functioning of thiamine in pyruvate utilization via acetyl-coenzyme A. Thus, an accumulation of pyruvate should lead to increased synthesis of alanine by transamination with glutamic acid and of serine via hydroxypyruvate and transamination (Ichihara and Greenberg, 1957). An experiment in which radioactive sodium formate was fed to normal and oxythiamine-inhibited roots provided additional evidence for this hypothesis. Examination of the results given in Table 5 shows that oxythiamine inhibition led to greater accumulation of C^{14} label in alanine and serine than in untreated roots. Treatment also led to the appearance of less radioactivity in malic and considerably less in citric acid than in untreated roots.

The most striking effect of desoxypyridoxine on free amino-acids in pond pine roots was the disappearance of γ-aminobu-

tyric acid, which was present in all roots except those receiving this treatment. Lack of γ-aminobutyric acid constituted evidence for the interference of desoxypyridoxine with pyridoxal phosphate metabolism, either in the decarboxylation of glutamic acid in the formation of γ-aminobutyric acid, or in the transamination of succinic semialdehyde if γ-aminobutyric acid is formed via that pathway.

Two amino acid antimetabolites were tested. β-2-thienylalanine, an antimetabolite of phenylalanine, inhibited growth about 75 percent when added at a concentration of 10 ppm; and canavanine (0.1 ppm), an analogue of arginine, reduced growth about 60 percent. The effects of these antimetabolites on the free amino acids in pond pine roots were too extensive to be readily accountable. β-2-thienylalanine reduced the amounts of all amino acids except glutamic and aspartic acids. Canavanine inhibition resulted in a decrease in the amount of arginine, while γ-aminobutyric acid, threonine, and β-alanine increased conspicuously. Arginine was usually the most abundant free amino acid of isolated pond pine roots. Free β-alanine, however, was not detected under any experimental conditions except in canavanine-inhibited roots. Threonine was usually present only in trace amounts.

Evidence has been obtained by other investigators (see Fries, 1954) that growth inhibitory effects of canavanine are specifically counteracted by arginine. Therefore, the decrease in arginine observed in canavanine-inhibited pond pine roots was anticipated. The mechanisms leading to accumulation of β-alanine and threonine still remain obscure. When radioactive glucose, glutamic acid, and aspartic acid were supplied in tracer amounts to canavanine-inhibited roots, the carbon-14 label did not appear in β-alanine or threonine. Relative amounts of radioactivity in γ-aminobutyric acid did increase, however, especially when glutamic-U-C^{14} acid was fed.

TABLE 5. Relative radioactivity of compounds formed during metabolism of sodium formate-C^{14} by isolated pond pine roots inhibited by oxythiamine.

Compound in roots	Treatment of roots	
	Control	Oxythiamine-inhibited
	(Activity as percentage of total)	
Aspartic acid	3	2
Glutamic acid	4	3
Serine	15	25
Asparagine	....	3
Alanine	4	14
Glutamine	6	6
Arginine	8	....
Citrulline	2	2
Proline	3	2
γ-aminobutyric acid		2
Citric acid	12	2
Malic acid	25	10
Succinic acid	3	19
Phosphorylated compounds	5	3
Unknowns	10	7

Studies with radioactive metabolites. As a general means of learning about some of the most conspicuous features of metabolism, experiments with tracer amounts of some of the more common metabolites can be of real value. In the experiments reported here the roots were incubated for 48 hours in the presence of glucose-U-C^{14}, sodium formate-C^{14}, glutamic-U-C^{14} acid, aspartic-2,3-C^{14} acid, and succinic-2,3-C^{14} acid. The data given in Table 6 provide only a broad picture of pond pine root metabolism. Much more is to be learned from short-time experiments, degradation studies and determinations of specific activities. The data do, nevertheless, provide a basis for comparisons with radioactive labeling patterns in other plant material.

As shown in Table 6, the common amino acids and members of the organic acid cycle were well labeled from all of the radioactive substrates. Much of the radioactivity from glucose appeared in phosphorylated compounds, which were not

TABLE 6. Relative radioactivity of compounds formed during metabolism of radioactive substrates by isolated pond pine roots.

Compound in roots	Compound supplied: Glucose-U-C^{14}	Sodium formate-C^{14}	Glutamic-U-C^{14} acid	Aspartic-2,3-C^{14} acid	Succinic-2,3-C^{14} acid
	(Activity as percentage of all compounds on paper exclusive of those supplied)				
Aspartic acid	17	3	1	--	1
Glutamic acid	6	4	--	9	8
Serine	[1]	15	1	--	--
Glycine	[1]	--	--	--	--
Asparagine	--	--	16	20	9
Alanine	11	4	2	3	8
Glutamine	3	6	13	39	22
Arginine	--	8	--	--	--
Citrulline	--	2	2	--	1
Proline	3	3	--	--	1
γ-aminobutyric acid	5	--	43	7	13
Citric acid	2	12	4	2	1
Malic acid	3	25	6	8	18
Succinic acid	2	3	1	1	--
Fumaric acid	--	--	--	--	9
Glucose	[1]	--	--	--	--
Fructose	8	--	--	--	--
Sucrose	11	--	--	--	--
Phosphorylated compounds	25	5	4	--	2
Unknowns	4	10	7	11	7

[1]Glucose, serine, glycine were not separated on the radioautogram.

identified but occupied chromatographic positions characteristic for intermediates of glycolysis. Sodium formate was apparently utilized via at least three pathways: (1) incorporation as a "C_1" fragment into serine; (2) fixation via malic acid; and (3) fixation via ornithine into citrulline and arginine. Further evidence for the latter fixation pathway will be given in a future paper. Radioactive glutamic acid was rapidly converted into γ-aminobutyric acid, possibly through glutamic decarboxylase activity. Label from aspartic-2,3-C^{14} acid appeared mainly in glutamine and asparagine after 48 hours' incubation. Succinic-2,3-C^{14} acid was not metabolized as rapidly as the other compounds. Presumably, penetration into the cells was slow. Glutamine and γ-aminobutyric acid, among the amino acids, became heavily labeled from radioactive succinate.

Amino acid utilization experiments. In another series of experiments the roots were used in much the same manner as bacteria or perfused animal organs in studying amino acid metabolism. The basic rationale was that substances normally derived from a given metabolite will accumulate to a sufficient degree to be readily detectable if appreciable quantities of the metabolite in question are supplied to the tissue.

Non-radioactive amino acids (10 ppm) were added to the medium and the resulting changes in free amino acids in the

TABLE 7. Apparent effects of amino acids (10 ppm), when added singly to medium, on growth of pine roots.

Strongly inhibitory	Slightly inhibitory	No apparent effect	Stimulatory
β-alanine	Lysine	Alanine	Aspartic acid
Cysteine	Methionine	Asparagine	Arginine
Glycine		Glutamine	Citrulline
Homocystine		Histidine	Glutamic acid
Hydroxyproline		Tryptophan	Ornithine
Leucine		Tyrosine	Glutathione
Serine			Proline
Threonine			

roots were followed chromatographically. General growth responses, inhibition or stimulation, were also noted and recorded. Some 23 amino acids were tested in this manner (see Table 7), and a number of noteworthy results were obtained. These may be briefly summarized.

Glutamic acid, glutamine, and glutathione additions to the nutrient solution each resulted in an increase in γ-aminobutyric acid in the tissue. Supplying cysteine resulted in increases in threonine and alanine, while homocystine in the nutrient solution led to marked increases in amounts of free alanine and serine in the tissue. Glycine and methionine additions resulted in large accumulations of serine; but reactions leading to serine formation were seemingly not readily reversible because the addition of serine did not cause an increase in free glycine or methionine. On the addition of ornithine there was an increase in the amount of citrulline. Numerous other, less conspicuous, changes in free amino acids were noted. It was apparent that isolated roots were very useful as test material in studying amino acid metabolism.

Discussion

Roots of pond pine, and of four other pine species, have been cultured successfully in a medium similar to that developed by Slankis (1947, 1948a, 1950) and improved by Isikawa (1956). The optimum concentration of sucrose for growing pond pine was found to be 8 percent; whereas, according to Slankis, Scotch pine roots grew best with 7 percent sucrose. Isikawa (1956) reported that adding pyridoxine and nicotinic acid to Slankis' medium improved the growth of the roots of Japanese red pine, and essentially the same results were found with those of pond pine. Absence of light was essential in maintaining growth. Pfeffer (1903) cited several instances where light had been shown to influence root shape, branching habit, and growth rate. More recent studies (Torrey, 1952) on certain isolated roots have confirmed the earlier observations. Isolated roots of the five pine species studied by us, however, appear to be more absolute in their dark requirement than any others previously reported.

Superiority of Slankis' medium to White's was shown to be largely due to the lack of glycine. Other work with isolated roots has indicated that any of a number of amino acids, when added singly to the medium, inhibit growth. Inhibition of growth of excised groundsel roots by glycine, as reported by Skinner and Street (1954) was reversible by adding arginine. The same was found to be true for pond pine roots. The mechanism for the reversal of glycine inhibition has not been found; neither has the mechanism of glycine inhibition been established. But one may infer from the present work with pond pine roots that excess accumulation of serine may be responsible for the inhibition noted when glycine was added.

Pond pine roots were apparently able to synthesize most of the vitamins required for growth. It has been shown that isolated roots of most species do in fact synthesize vitamins, although one or more may be in suboptimal amounts making it necessary to add them to the nutrient medium. Reversible inhibitions of growth of pond pine roots by oxythiamine and desoxypyridoxine

were demonstrated. The mechanism of inhibition by oxythiamine appeared to be through interference with pyruvate utilization, as would be expected from known functions of thiamine in metabolism. Similarly, chromatographic evidence was obtained that desoxypyridoxine interfered with the functioning of pyridoxal phosphate in decarboxylation or transamination reactions of amino acids.

Modifications of branching of pond pine roots were caused by a number of apparently unrelated compounds. Although the work of Slankis (1948b, 1949b, 1950, 1951) indicated that IAA and NAA were effective in causing dichotomous branching of Scotch pine roots, other compounds (kinetin, amino acids, vitamins) were found to be more effective with pond pine. It is not known whether Slankis tested compounds other than auxins, but the present work with pond pine indicates that the physiological basis for dichotomous branching of pine roots is quite complex.

Isolated pond pine roots proved to be very useful test objects in studying metabolic pathways. This was particularly true in determining changes in amino acids resulting from feeding inhibitors, antimetabolites, and amino acids. Evidence from amino acid feedings and radioactive tracer studies indicated the possible operation of an ornithine-urea cycle, which is being investigated further. Numerous other results are also being subjected to more exacting experimentation.

Summary

1. Isolated roots of pond pine (*Pinus serotina* Michx.), sand pine (*Pinus clausa* (Chapm.) Vasey), longleaf pine (*Pinus palustris* Mill.), slash pine (*Pinus elliottii* Englm.) and loblolly pine (*Pinus taeda* L.) were successfully grown in sterile culture and maintained in subculture for more than two years.

2. Light proved to be inhibitory to growth of roots of all five species.

3. White's medium was inferior to Slankis' medium; but if the glycine were removed from White's medium, growth of roots was satisfactory. Glycine inhibition was reversible by adding arginine. Serine, formed in large amounts from glycine in pond pine roots, was apparently the inhibiting compound rather than glycine per se.

4. Eight percent sucrose was the best carbon source tested.

5. Investigations of reversible inhibitions of growth by oxythiamine and desoxypyridoxine helped elucidate some of the functions of thiamine and pyridoxine in pond pine roots.

6. Numerous compounds including kinetin, auxins, vitamins, and amino acids caused changes in the branching habit of the roots. Simulation of the dichotomous branching habit normally associated with mycorrhizal development was induced by a variety of treatments.

7. Respiratory inhibitors, antimetabolites, and radioactive metabolites, in conjunction with paper chromatography, proved effective in outlining some of the major metabolic pathways in the roots.

8. Alterations in free amino acid patterns resulting from various treatments showed that isolated roots were excellent material for studies of amino acid metabolism.

Literature Cited

Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas, and W. Stepka. 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J. Amer. Chem. Soc. 72: 1710-1718.

Block, R. J., E. L. Durrum, and G. Zweig. 1958. A Manual of Paper Chromatography and Paper Electrophoresis. 2nd ed., Academic Press, Inc., 1958. 710 pp.

Bonner, J. 1942. Culture of isolated roots of *Acacia melanoxylon*. Bull. Torrey Bot. Club. 69: 130-133.

FRIES, N. 1954. Chemical factors controlling the growth of the decotylised pea seedling. Symb. Bot. Upsaliensis 13: 1-83. (see page 57)

ICHIHARA, A. AND D. M. GREENBERG. 1957. Further studies on the pathway of serine formation from carbohydrate. J. Biol. Chem. 224: 331-340.

ISIKAWA, H. 1956. Eine präliminare Mitteilung über das Wachstum der isolierten Wurzeln von *Pinus densiflora* und *Pinus Thunbergii*. J. Jap. For. Soc. 38: 5-7.

PFEFFER, W. 1903. The Physiology of Plants. 2nd ed. Translated by A. J. Ewart, Vol. 2, Oxford Press, 296 pp. (See pages 87, 93).

SEELIGER, I. 1956. Uber die Kultur isolierter Wurzeln der Robinie (*Robinia pseudoacacia* L.). Flora 144: 47-83.

SKINNER, J. C. AND H. E. STREET. 1954. Studies on the growth of excised roots. II. Observations on the growth of excised groundsel roots. New Phytol. 53: 44-67.

SLANKIS, V. 1947. Influence of the sugar concentration on the growth of isolated pine roots. Nature 160: 645-646.

————. 1948a. Verschiedene Zuckerarten als Kohlenhydratquelle für isolierte Wurzeln von *Pinus silvestris*. Physiol. Plant. 1: 278-289.

————. 1948b. Einfluss von Exudaten von *Boletus variegatus* auf die dichotomische Verzweigung isolierter Kiefernwurzeln. Physiol. Plant. 1: 390-400.

————. 1949a. Einfluss der Temperatur auf das Wachstum der isolierten Wurzeln von *Pinus silvestris*. Physiol. Plant. 2: 131-137.

————. 1949b. Wirkung von β-indolyessigsäure auf die dichotomische Verzweigung isolierter Wurzeln von *Pinus silvestris*. Svensk. Bot. Tidskr. 43: 603-607.

————. 1950. Effect of α-naphthaleneacetic acid on dichotomous branching of isolated roots of *Pinus silvestris*. Physiol. Plant. 3: 40-44.

————. 1951. Uber den Einfluss von β-indolyessigsäure und anderen Wuchsstoffen auf das Wachstum von Kiefernwurzeln. Symb. Bot. Upsaliensis. 11(3): 1-63.

STREET, H. E. 1957. Excised root culture. Biol. Revs. 32: 117-155.

TORREY, J. G. 1952. Effects of light on elongation and branching in pea roots. Plant Physiol. 27: 591-602.

WHITE, P. R. 1943. A Handbook of Plant Tissue Culture. Jacques Cattell Press, Lancaster, Penna. 277 pp.

————. 1951. Nutritional requirements of isolated plant tissues and organs. Ann. Rev. Plant Physiol. 2: 231-244.

WOOLLEY, D. W. 1952. A Study of Antimetabolites. John Wiley and Sons, Inc. New York. 269 pp.

The Leuco-anthocyanin Distribution In the Genus Pinus

BY
STANLEY L. KRUGMAN

THE PRECISE ROLE that leuco-anthocyanins play in plant metabolism is not known, although it has been suggested that this group of compounds is the precursor of the anthocyanins (Rutzler, 1939; Alston and Hagen, 1955). Hillis (1956) believes that the leuco-anthocyanins may be precursors of the bark and heartwood extractives in *Eucalyptus*, while Steward and Shantz (1955) conclude that the growth promoting activity of immature *Aesculus* fruit could in part be attributed to a leuco-anthocyanin.

Although the distribution of the leuco-anthocyanins both in woody and herbaceous plants has been studied in some detail, little is known concerning their distribution in the genus *Pinus* (Robinson and Robinson, 1933; Lawrence *et al.*, 1939; Rutzler, 1939; Bate-Smith, 1954; Bate-Smith and Lerner, 1954). Robinson and Robinson (1933) were the first to report the presence of a leuco-anthocyanin in the wood of pine.[1] Subsequently, Bate-Smith (1954) noted its presence in the wood of the Maritime pine (*Pinus pinaster* Ait.), while Hillis (1954) has reported its presence in Monterey pine (*Pinus radiata* Don.). In all of these instances the leuco-anthocyanins yielded cyanidin upon acid hydrolysis. Hillis (1954) also reported the presence of both a cyanidin and delphinidin-yielding leuco-anthocyanin in Monterey pine bark. Robinson and Robinson (1934) reported finding leuco-anthocyanins in the cones of the Himalayan white pine (*Pinus excelsa* Wallich.) and Scotch pine (*Pinus sylvestris* L.), and Krugman (1956) reported finding leuco-anthocyanins in the embryo and seedlings of sugar pine (*Pinus lambertiana* Dougl.). In the latter instance the leuco-anthocyanin yielded cyanidin and delphinidin upon acid hydrolysis.

In view of the suggestion that the leuco-anthocyanins may serve as the precursors of the anthocyanins, flavonoids, and tannins, the presence of the leuco-anthocyanins within the genus *Pinus* and their distribution within the individual parts of the pine is of considerable interest. With this in mind several of the native and exotic pines were analyzed for the presence of leuco-anthocyanins in their tissues. The results are reported here.

Materials and Methods

Source and preparation of plant material for analysis. The root, bark, and wood samples investigated were taken from pines growing on the Berkeley campus of the University of California and from pines of the Eddy Arboretum at the Institute of Forest Genetics at Placerville. The pollen

[1]The species name was not indicated.

The author is a graduate student at the School of Forestry, University of California, Berkeley.

samples were supplied by the Division of Forest Genetic Research, California Forest and Range Experiment Station.

In all instances the materials analyzed were free of any anthocyanin pigmentation and with the exception of the pollen samples, all analyses were carried out within two hours of the time of collection. The latter condition was considered necessary in view of the report by Hillis (1956) that the leuco-anthocyanin content may decrease during storage.

From each pine under investigation several live branches containing the current year's growth (1955 or 1956) were selected and 3 to 4 grams (fresh weight) of healthy green needles were removed. Care was taken not to include the fascicle sheaths since they had a definite reddish coloration which would interfere with the identification of the leuco-anthocyanin. To ensure complete pigment extraction the needles were thoroughly ground in a mortar with the extracting solvent prior to heating.

The same branches from which the needles were selected were also used as the source of the bark and wood samples. The bark was carefully peeled from the wood (xylem and pith), and following the removal of fragments of the fascile sheaths, was cut by hand into a number of segments and extracted as described in the next section.

Approximately 5 grams (fresh weight) of the remaining wood were split into fragments and then crushed with the extracting solvent in a mortar. The crushed wood and the extracting solvent were then heated.

The root material was taken from seedlings of varying ages up to 3 years, which had been grown from known seed sources. The roots were thoroughly washed upon removal from the intact plant, and cut into a number of smaller segments before extraction. The phloem was not separated from the xylem prior to the extraction as was the case for the aerial stem.

The pollen grains were either extracted with the solvent directly with no prior treatment, or were ground with a fine sand and then treated with the extracting solvent.

Identification of the leuco-anthocyanins. The leuco-anthocyanins were characterized by the anthocyanidin produced when hydrolyzed with strong acid.

Three to four grams of cut or crushed plant material were boiled in 2N aqueous hydrochloric acid for 30 minutes. During this period the colorless leuco-anthocyanins were converted into the colored anthocyanidins. The colored solution was then filtered through a fritted disk funnel. The filtered solution was then placed in a separatory funnel and 5 cc of iso-amyl alcohol added. After shaking the separatory funnel, the red color, which was for the most part caused by anthocyanidins, appeared in the upper iso-amyl layer.

The anthocyanidins were then further purified by washing the iso-amyl alcohol layer four times with an excess of 1 percent hydrochloric acid. This removed a number of the readily water-soluble extractives, but not the anthocyanidins. To the washed iso-amyl alcohol layer a three-fold volume of benzene was added, and then to this one phase system of benzene and iso-amyl alcohol, 5 cc of 1 percent hydrochloric acid was added. The solution was shaken vigorously, which caused the anthocyanidins to be removed from the organic phase into the lower aqueous acid phase. The anthocyanidin pigments were re-extracted from the aqueous acid solution by the addition of 2 cc of iso-amyl alcohol. By this re-extraction and washing most of the colored impurities were removed from the anthocyanidin solution (Geissman, 1955).

The unknown anthocyanidins were then identified by the paper chromatographic procedure described by Krugman (1956).

In the experiments in which different pine pollens were analyzed the pollen grains after being ground with sand and boiled with 2N hydrochloric acid for 30

minutes did not develop a color that could be attributed to the conversion of the leuco-anthocyanins to anthocyanidins. Therefore, a second method was used in which the pollen grains were ground with fine sand in 5 cc of 90 percent methanol. Following the filtering of the ground pollen material, 5 drops of concentrated hydrochloric acid were added to the methanol filtrate and the solutions thoroughly mixed. Then to this acid methanol extract 6 drops of a saturated solution of vanillin in 95 percent ethanol were added. The lack of any color development with the addition of the vanillin indicated the absence of any leuco-anthocyanins in the pollen.

Results and Discussion

The pine species investigated for the presence of leuco-anthocyanins are listed in Table 1. Leuco-anthocyanins which yielded cyanidin and delphinidin upon acid hydrolysis were detected in all pines analyzed. This agrees with the results of Bate-Smith (1954) and Bate-Smith and Lerner (1954), who investigated a number of woody species and found only cyanidin and delphinidin - yielding leuco - anthocyanins. However, the leuco-anthocyanin content does vary among the different parts of the pine investigated. The needles and bark of all species tested contained leuco-anthocyanins which yielded both cyanidin and delphinidin upon acid hydrolysis, while the roots and stem wood contained only cyanidin yielding leuco-anthocyanins.

Initially, it appeared that the leuco-anthocyanins which yielded delphinidin in the roots and stem wood were in too low a concentration to be detected in the samples; but, even when 10 to 20 gram samples were used the results were the same. Only the leuco-anthocyanins which yielded cyanidin could be detected.

To determine whether or not the lack of delphinidin yielding leuco-anthocyanins in the roots and stem wood was a seasonal phenomenon, monthly samples of roots and stem wood from sugar and ponderosa pine were analyzed chromatographically throughout the year for their leuco-anthocyanin content. In every instance only leuco-anthocyanins which yielded cyanidin could be detected.

In contrast to these results with pine where only cyanidin yielding leuco-anthocyanins were found in the woody tissue, Hillis (1956) reported that leuco-anthocyanins which yielded both cyanidin and delphinidin were readily detected in the sapwood and heartwood of *Eucalyptus sieberiana*. However, both the bark and leaves of Eucalyptus also contained leuco-anthocyanins which yielded cyanidin and delphinidin, a condition which is similar to that found among the pines studied.

Although the leuco-anthocyanins are readily detected in the stem wood, bark, and needles, they were not detected in the pollen grains. They also appear to be lacking in the female gametophytic tissue of sugar pine (Krugman, 1956).

Investigations in which only the leaves or needles were analyzed for leuco-anthocyanins indicated no qualitative differences in leuco-anthocyanin content (Rutzler, 1939; Bate-Smith, 1954). When other parts of the plant such as the root or stem wood are analyzed as in the present study on pines, definite differences do exist in the presence of leuco-anthocyanins. Although the significance of these differences is not fully apparent at this time, any future evaluation of the leuco-anthocyanins as the precursor of the flavonoids, tannins and anthocyanins in pine must reconcile these differences in distribution.

Literature Cited

Alson, R. E. and C. W. Hagen. 1955. Relation of leuco-anthocyanins to anthocyanin synthesis. Nature 175: 990.

Bate-Smith, E. C. 1954. Leuco-anthocyanins 1. Detection and identification of anthocyanins formed from leuco-anthocyanins in plant tissue. Biochem. J. 58: 122-125.

——— and N. H. Lerner. 1954.

TABLE 1. Species of the genus Pinus in which the leuco-anthocyanins were investigated. Asterisk (*) indicates the plant part analyzed.

Species	Plant part studied				
	Needles	Bark	Wood	Roots	Pollen grains
Sub-genus *Haploxylon*					
cembroides Zucc.	*	*	*		
excelsa Wallich.	*	*	*		
flexilis James	*	*	*		
lambertiana Dougl.	*	*	*	*	*
monticola Dougl.	*	*	*		
monticola × *strobus*	*	*	*		
strobus L.	*	*	*	*	
Sub-genus *Diploxylon*					
attenuata Lemm.	*	*	*		
× *attenuradiata* Stockwell and Righter	*	*	*		
banksiana Lamb.	*	*	*		
× *murraybanksiana* Righter and Stockwell	*	*	*		
canariensis Smith	*	*	*		
caribaea Morelet	*	*	*		*
contorta Dougl.	*	*	*	*	
coulteri D. Don	*	*	*		
densiflora Sieb. and Zucca.	*	*	*		*
echinata Miller	*	*	*		
jeffreyi Grev. & Balf.	*	*	*	*	*
glabra Walt.	*	*	*		
halepensis Mill.	*	*	*		*
leiophylla Schect. and Cham.	*	*	*		
montana Mill.	*	*	*		
montezuma Lindl.	*	*	*		
muricata D. Don.	*	*	*		*
murrayana Balf.	*	*	*		
nigra Arnold	*	*	*		
palustris Mill.	*	*	*		*
× *sondereggeri* Chapm.	*	*	*		
patula Schl. and Chapm.	*	*	*		*
pinaster Ait.	*	*	*		
pinea L.	*	*	*		
ponderosa Laws.	*	*	*	*	*
ponderosa var. *scopulorm* Engelm.	*	*	*		*
ponderosa × *engelmannii*	*	*	*		
ponderosa × *jeffreyi*	*	*	*		
pseudostrobus Lindl.	*	*	*		
radiata D. Don.	*	*	*	*	*
sabiniana Dougl.	*	*	*	*	
serotina Michx.	*	*	*		
sylvestris L.	*	*	*		
taeda L.	*	*	*		
thumbergii Parl.	*	*	*		*
torreyana Parry	*	*	*		*
washoensis Mason & Stockwell	*	*	*		

Leuco-anthocyanins 2. Systematic distribution of leuco-anthocyanins in leaves. Biochem. J. 58: 126-132.

GEISSMAN, T. A. 1955. Modern methods of plant analysis. Anthocyanins, chalcones, aurones, flavones and related water-soluble plant pigments. Vol. 3 (Ed. K. Paek and M. V. Tracey). Springer-Verlag, Berlin. pp. 450-496.

HILLIS, W. E. 1954. The precursors of the red color in leather. Preliminary survey. J. Soc. Leather Trades Chem. 38: 91-102.

————. 1956. Leucoanthocyanins as the possible precursors of extractives in woody tissues. Aust. J. Biol. Sci. 9: 263-280.

KRUGMAN, S. 1956. The anthocyanin and leuco-anthocyanins of sugar pine seedlings. For. Sci. 2: 273-280.

LAWRENCE, W. J. C., J. R. PRICE, G. M. ROBINSON and R. ROBINSON. 1939. The distribution of anthocyanins in flowers, fruit and leaves. Phil. Trans. Roy. Soc. London. 230: 149-178.

ROBINSON, G. M. and R. ROBINSON. 1933. A survey of anthocyanins III. Notes on the distribution of leuco-anthocyanins. Biochem. J. 27:206-212.

————. 1934. A survey of anthocyanins IV. Biochem. J. 28: 1712-1720.

RUTZLER, J. E. 1939. The precursors of the anthocyanins of autumn foliage. J. Am. Chem. Soc. 61: 1160-1163.

STEWARD, F. C. and F. M. SHANTZ. 1955. The chemistry and mode of action of plant growth substances. The chemical induction of growth in plant tissue cultures. (Ed. R. L. Wain and F. Wightman.) Academic Press, New York. pp. 165-186.

First Forest Science Monograph Issued

The first *Forest Science Monograph* entitled "Private Forestry in Norway—A Case Study in Small Woodland Management and Policy" by John A. Zivnuska is being mailed concurrently with this issue of FOREST SCIENCE.

Forest Science Monographs are distributed to all subscribers of FOREST SCIENCE without extra charge. No separate subscription is required.

Germination of Douglas-Fir Seed as Affected by Light, Temperature, and Gibberellic Acid

BY
S. D. RICHARDSON

NUMEROUS STUDIES have been made of light and temperature as factors in seed germination (Evanari, 1955; Toole *et al.*, 1956); but in the extensive literature concerning these factors, data relating to tree species are conspicuously deficient. Some attention has been paid to the establishment of optimal temperature conditions for germination (Baldwin, 1942; Edwards, 1932), but with a few notable exceptions[1] critical studies of the interaction between light and temperature in tree seed germination are lacking.

With regard to gibberellic acid the picture is, not surprisingly, similar. It has been observed that germination in certain herbaceous species can be accelerated by treatment with gibberellic acid (Stowe and Yamaki, 1957), and recently the relation between such acceleration and the red-infrared light effect in lettuce seeds has been investigated (Kahn *et al.*, 1956; Lona, 1956). It is obvious, however, that more data will be required before a significant contribution to an understanding of these interactions can be made.

The present report describes experiments which demonstrate that at temperatures below the optimum, germination of non-stratified seed of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is accelerated by exposure to long photoperiods of low intensity illumination; the action of gibberellic acid, however, seems to be independent of the light response.

Experimental Methods and Results

The seed used throughout these experiments was collected in 1955 from trees near the Van Duzen River, Humboldt County, California, and stored dry in polyethylene bags at a temperature of 5° C. Germination tests were made in 4½-inch petri dishes on 2 layers of Whatman No. 42 filter paper with either 20 or 25 seeds per dish. The experiments were all carried out in controlled environment rooms at the Earhart Plant Research Laboratory of the California Institute of Technology.

---

[1]Allen, 1941; Black and Wareing, 1955; Rohmeder, 1938; Stearns and Olson, 1958; Vaartaja, 1956.

The author is on the staff of the Department of Forestry, University of Aberdeen, Scotland. This work was done while he was on leave of absence and employed by the Forest Service, United States Department of Agriculture. Co-operators in the study are the Forest Service and the California Institute of Technology.

The author acknowledges with pleasure the help and encouragement given by James Bonner and Henry Hellmers during the course of the work.

Experiment 1. The first experiment was largely exploratory. A factorial design was used involving 4 replicates x 2 light treatments (continuous light at 4,000 lux from "daylight" type fluorescent lamps and continuous darkness) x 5 gibberellic acid treatments (0, 3, 10, 30 and 100 ppm applied in de-ionized water at a rate of 5 ml per dish at the beginning of the experiment and at a rate of 2 ml per dish at 14-day intervals thereafter) x 2 seed pre-treatments (stratified for 8 weeks in moist sand at 5° C and non-stratified). Complete randomization within light treatments was employed, and the experiment was run at a temperature of 20° C.

Experimental treatments began on February 12, 1957 and germination counts were made at 2- to 3-day intervals for a period of 46 days thereafter, the results being expressed as percentages of the maximum germination possible. Seeds treated with continuous darkness were examined in green light.

In the course of this experiment it was found that the rate of germination in non-stratified seed is markedly lower than that in stratified seed; but that exposure to light accelerates germination in non-stratified material. In an attempt to confirm the light effect, half the replicates in the non-stratified, dark-germinated series were transferred to continuous light 12 days after the start of the experiment. Material treated in this way has not been included in any of the statistical analyses.

Table 1 and Figures 1 and 2 summarize the results of this experiment. Table 1 gives the germination percent after 46 days according to treatment, the time required to reach 50 percent maximum germination, and the results of analyses of variance of these factors. Treatment differences in total germination (i.e., germination percent after 46 days) are not marked, but there are very highly significant differences in germination rates.

Figure 1 illustrates the effect of light and stratification on the time course of germination. It can be seen that stratification markedly accelerates germination but with stratified seed there is no effect of light treatment on the germination pattern. On the germination of nonstratified seed, on the other hand, light has a pronounced accelerating effect, illustrated most convincingly by a comparison of seeds remaining throughout the experiment in continuous darkness with those transferred to light after 12 days darkness.

Turning to the effects of gibberellic acid on the germination rate, Figure 2 shows the relation between concentration and the time required to reach 50 percent germination. There is slight but consistent evidence of a stimulation within the range of 3 to 10 ppm

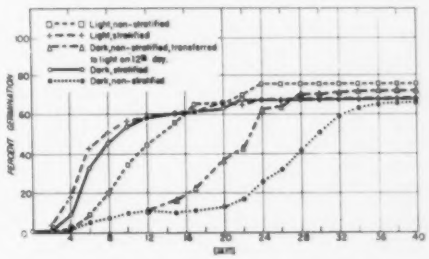

FIGURE 1. *Douglas-fir: The effect of light and and stratification on total germination.*

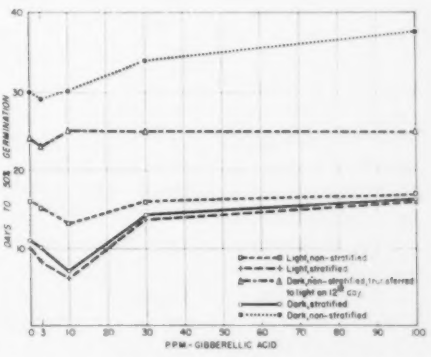

FIGURE 2. *Douglas-fir: The effect of gibberellic acid on speed of germination.*

TABLE 1. Douglas-fir. Rate and amount of germination according to pre-treatment, light, and gibberellic acid treatments. (Columns 4 and 5 show means of 4 replicates.)

Pre-treatment (1)	Germination condition (2)	Gibberellic acid concentration ppm (3)	Total germination percent (4)	Days to 50 percent germination (5)
Non-stratified	Light	0	75	16
		3	81	15
		10	78	13
		30	74	16
		100	77	17
Stratified	Light	0	72	10
		3	71	8
		10	74	6
		30	64	14
		100	59	16
Non-stratified	Dark	0	66	30
		3	74	29
		10	74	30
		30	62	34
		100	58	38+
Stratified	Dark	0	67	11
		3	71	10
		10	74	7
		30	65	14
		100	64	16
Non-stratified	Dark-light	0	70	24
		3	74	23
		10	82	25
		30	74	25
		100	60	25

Analyses of variance
Days to 50 percent germination

Source of variation	Degrees of freedom	Variance ratio
Light (L)	1	16.908***
Stratification (S)	1	62.946***
Gibberellic acid (G)	4	5.317**
L x S	1	54.529***
L x G	4	0.096
S x G	4	0.572
L x S x G	4	0.354
Within treatments (error term)	50	

TOTAL GERMINATION
(Data transformed to angles)

Source of variation	Degrees of freedom	Variance ratio
Light (L)	1	3.213
Stratification (S)	1	4.722*
Gibberellic acid (G)	4	2.020
L x S	1	2.577
L x G	4	0.121
S x G	4	0.192
L x S x G	4	0.428
Within treatments (error term)	50	

*** = Significant at 0.1 percent level.
** = Significant at 1.0 percent level.
* = Significant at 5.0 percent level.

and some sign of a depressive effect of concentrations above 10 ppm. The effect of gibberellic acid is statistically significant at the 1 percent level (Table 1).

There is some evidence too that the optimal concentration for accelerating germination is higher for stratified than non-stratified seed. This may be an illusion; the stratified seed was fully imbibed at the beginning of the experiment and it is to be expected that the imbibed moisture might have a diluting effect on the gibberellic acid applied and thus mask the effective concentration.

Experiment 2. In order to investigate the effect of gibberellic acid more precisely, an experiment was carried out using 6 concentrations of acid within the range of 0 to 10 ppm. The same light treatments were employed (continuous light at 4,000 lux and continuous darkness), but only non-

stratified seed was used. The temperature was 20° C and the experimental procedure followed that of the first experiment, except that germination counts were continued for 51 days after the beginning of the treatments.

Figure 3 summarizes the significant results of this series. Gibberellic acid accelerates germination in both light and dark-germinated seed, the effect being most marked at 5 ppm. There is no evidence of interaction between light and gibberellic acid but, as in the first experiment, the accelerating effect of light is the most striking feature; exposure to continuous illumination hastens rate of germination by about 23 days.

Experiment 3. The next experiment was designed to examine the nature of the light effect in more detail. Non-stratified seeds were germinated in dishes containing deionized water at 3 temperatures (14°, 20°, and 26° C) and under 4 light intensities (0, 520, 1,010, and 1,500 lux). Light intensity differences were achieved by covering the dishes with either lightproof paper or layers of white gauze; exposure during this experiment was limited to 16 hours per daily cycle and germination counts were made at 2- to 3-day intervals under a green light. Each treatment was replicated 4 times and randomization was within treatments.

Total germination percentages and germination rates are presented in Table 2 by treatment. It should be pointed out that at temperatures of 20° and 26° C, germination was regarded as complete after 47 days and germination counts were not made beyond this period; in the 14° C series, however, counts were continued until 82 days had elapsed from the start of the treatment.

At all light intensities total germination was some 10 percent lower at 14° C than at the other temperatures (Table 2). It is not clear whether this effect is real or not; by the time the experiment was terminated the seeds at 14° C were severely infected by fungi and probably many of the remaining ungerminated seeds were no longer viable. Values given for total germination at 14° C must, therefore, be regarded cautiously.

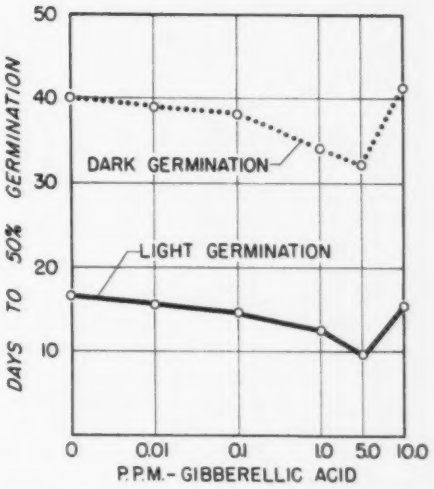

FIGURE 3. *Douglas-fir: The effect of light and gibberellic acid on germination of non-stratified seed (means of 4 replicates).*

The values relating to germination rate can be discussed more confidently. It is apparent that, relative to a temperature of 20° C, germination at 14° C is slow in both light and dark; at 26° C, relative to 20° C, on the other hand, germination in the dark is accelerated. At 26° C there is no effect of light on germination rate, while at the lower temperatures light markedly accelerates germination. These relations are illustrated in Figure 4 which shows germination rate relative to treatment. This figure also shows that, under the conditions of this experiment, the light reaction is saturated at the lowest of the intensities employed; there are no significant differences with regard to germination rate between 520, 1,010, and 1,500 lux.

As a corollary to this experiment in an attempt to estimate the intensity operative

TABLE 2. Douglas-fir. Rate and amount of germination of non-stratified seed according to light and temperature treatment. (Columns 3 and 4 show means of 4 replicates.)

Temperature °C (1)	Light intensity (lux) (2)	Total germination (3)	Days to 50 percent germination (4)
14	0	55	74
	520	63	61
	1010	68	59
	1500	58	60
20	0	70	29
	520	71	17
	1010	75	18
	1500	70	18
26	0	73	17
	520	76	19
	1010	74	17
	1500	79	15

Analyses of variance

Days to 50 percent germination

Source of variation	Degrees of freedom	Variance ratio
Temperature (T)	2	798.810***
Light (L)	3	13.927***
T x L	6	6.296***
Replicates	36	

TOTAL GERMINATION (Data transformed to angles)

Source of variation	Degrees of freedom	Variance ratio
Temperature (T)	2	10.556***
Light (L)	3	0.681
T x L	6	0.530
Replicates	36	

*** = Significant at 0.1 percent level.
** = Significant at 1.0 percent level.
* = Significant at 5.0 percent level.

in the light reaction, measurements of light transmission of seed coats were made. Dry seeds were split transversely, the cotyledons removed, and the optical density of "upper" (dark in color and markedly convex) and "lower" (light in color and somewhat flattened) coats measured with a Welch Densichron over a range of incident intensities from 100 to 50,000 lux. The coats were then soaked in de-ionized water for 3 hours (to simulate imbibition) and remeasured. They were then placed on filter paper in a current of dry air and remeasured after respectively 3 and 24 hours. The optical density of the seed coats was expressed as the percentage transmission of the incident light. The results of these measurements will not be presented in detail here. The tabulation which follows gives the mean values of 6 individual seeds measured at an incident light intensity of 500 lux.

Treatment	*Side*	*Mean percent of light transmitted*
Dry	Upper	19.05
	Lower	17.41
Imbibed	Upper	24.99
	Lower	16.21
Redried 3 hours	Upper	24.96
	Lower	16.30
Redried 24 hours	Upper	24.84
	Lower	16.26

About 20 percent of the incident light is transmitted by the upper seed coat in a dry condition and about 25 percent after soaking in water. It is of interest that drying for up to 24 hours does not significantly diminish this increased light permeability. The lower seed coats transmit slightly less light (17 percent) when dry and show no increase in permeability when soaked in water.

Experiment 4. The results of the last experiment made it clear that exposure to continuous light is not essential for accelerated germination in non-stratified seeds of Douglas-fir. Obvious interest attached to the question of how much light is required and at what stage in the germination process it is effective. In an attempt to answer these questions, in Experiment 4 seeds were exposed to light at various times and for various periods during and after a 3-hour period of imbibition with de-ionized water. The light exposure was at 3,000 lux and the temperature throughout was 18° C.

There were 3 series in which a single light exposure, ¼, 2¾, and 3 hours duration, was given during the period of imbibition; 5 series in which a single light exposure, 5 and 20 minutes, and 1, 8, and 16 hours, was given immediately following imbibition; 3 series in which exposure to light after imbibition was periodic (8, 16, and 24 hours per 24-hour cycle); and a dark control which received no light. Germination counts were made as in previous experiments and the experiment continued for 51 days.

The only treatments effective in accelerating germination over the dark control were the two long-day treatments of 16 and 24 hours. In these, the time required for 50 percent germination was reduced by some 20 days.

Discussion

The results of these preliminary experiments contain a number of features which merit comment. The accelerating effect of light on the germination of unstratified seed of Douglas-fir accords with the findings of Allen (1941) though he also found such an effect with stratified seed. In his experiments, the influence of light was greatest at high temperatures, while in the present work the reverse appears to be the case. Since, however, Allen was studying the influence of fluctuating temperatures and, further, the light conditions of his experiments were uncontrollable, strict comparison with the results presented here is scarcely justified. It is well known that the sensitivity of seeds to radiation depends upon temperature and can, indeed, be changed by suitable temperature treatments (Toole *et al.*, 1955). Apparently it can also be changed by stratification.

Photoperiodic responses of tree seeds are less well substantiated in the literature. Black and Wareing (1955) found an accelerating effect of continuous illumination on the germination of *Betula pubescens*, while Stearns and Olson (1958) report an interaction of photoperiod and temperature in unstratified seed of hemlock. They found that the optimum photoperiod for germination at 17° to 22° C is 8 or 12 hours; at 27° C it is 16 hours. The present finding that only exposure to at least 16 hours of light per daily cycle is effective in acceler-

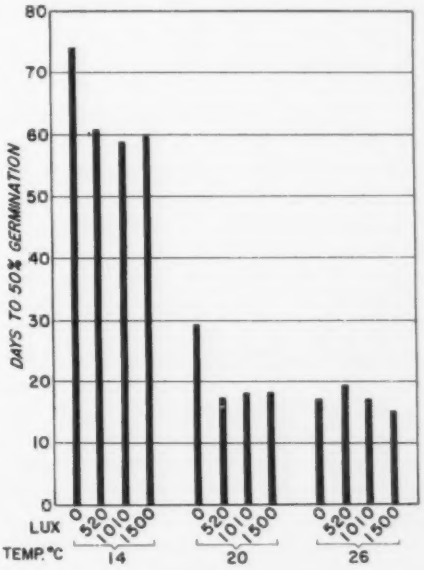

FIGURE 4. *Douglas-fir: The effect of temperature and light intensity on germination rate of non-stratified seed (means of 4 replicates).*

ating germination indicates that Douglas-fir corresponds more closely to birch than to hemlock though, again, comparisons with previous work cannot be made with any degree of confidence.

With regard to the intensity of exposure, little can be said since the site of the light-absorbing pigment is not known. If it is in the seed coat, measurements of light transmission are irrelevant; if, on the other hand, it is in the embryo the data presented here indicate that the reaction is saturated at 125 lux (= 650 ergs/cm^2/sec)—20 percent of the lowest intensity employed in Experiment 3. This intensity is well above that regarded by Black and Wareing (1955) as saturating the photoperiodic reaction in unstratified birch seed, or that required to break dormancy in intact buds of *Fagus sylvatica* (Wareing, 1953). It is of interest that the observed increase in light transmission of the seed coats upon soaking does not diminish when the seed coats are dried. This implies either that a permanent structural change occurs during soaking, or that something is leached out of the coat; the latter suggestion seems the most probable.

Effects of gibberellic acid on germination of Douglas-fir have not previously been investigated, though responses by some herbaceous species have been reported (Stowe and Yamaki, 1957). It seems to be generally agreed that gibberellic acid can accelerate germination rate but has no effect on total germination, as has been found in the present experiments.

Kahn *et al.* (1956) and Lona (1956) report that gibberellic acid can substitute for red light in promoting lettuce seed germination but that its effect is not reversed by infrared radiation; and, further, that it does not substitute for red light after high temperature treatment. This, as Stowe and Yamaki point out, indicates that gibberellic acid does not act specifically on the light receptor but rather as a general germination stimulant. Lang[2] believes that in germination it serves to block the infrared effect (which, in light-germinating lettuce seed, is responsible for the red light requirement) but does not interact with light *per se*. These views are supported by the observation reported in the present paper that the effect of gibberellic acid on germination of Douglas-fir is quantitatively similar in both dark and light-germinated seed.

The physiological significance of the present results cannot yet be evaluated. In spite of the considerable literature relating to germination studies the process is very imperfectly understood. Toole *et al.* (1956) point out that germination is controlled by a complex "system of interlocking reactions that must be kept in proper balance." As far as tree seeds are concerned, it is only very recently that some of the components of the system have been recognized; and, while there is no reason to suppose that woody species are physiologically any different from herbaceous plants in this respect, the accumulation of more data —especially with regard to light effects— must precede any attempt to translate recognition into understanding.

[2]Personal communication from A. Lang.

Summary

A study of the effects of light, temperature, and gibberellic acid on germination of Douglas-fir seed in a controlled environment demonstrated that the germination of nonstratified seed can be accelerated by up to 23 days (50 percent germination) by exposure to long photoperiods (16 or 24 hours light per daily cycle) at temperatures of 14° or 20° C, but not at 26° C. Total germination, however, is unaffected. The effect appears to be a genuine long-day phenomenon in that the reaction is light-saturated at 520 lux and cannot be effected by light treatments of shorter duration (8 hours per day, or single exposures prior to, during, or after imbibition).

Measurements of light transmission of the seed coats with a Welch Densichron over a range of incident intensities from 100 to 50,000 lux showed that about 20

percent of the incident light is transmitted by the upper seed coat in a dry condition and about 25 percent after soaking for 3 hours in water. Subsequent drying for 24 hours does not diminish the increased light permeability. Lower seed coats transmit 17 percent of the incident light and show no increase in permeability after soaking.

Gibberellic acid at 5 ppm accelerates germination by up to 7 days in both light and dark but, again, does not affect total germination. This is regarded as confirming previous indications that, in germination, gibberellic acid does not interact with light but acts as a general germination stimulant.

Literature Cited

ALLEN, G. S. 1941. Light and temperature as factors in the germination of the seed of Douglas-fir. For. Chron. 17: 99-109.

BALDWIN, H. I. 1942. Forest tree seed of the north temperate regions. Chronica Botanica Co., Waltham, Mass. 240 pp.

BLACK, M., and P. F. WAREING. 1955. Growth studies in woody species. VII. Photoperiodic control of germination in *Betula pubescens*, Ehrh. Physiol. Plant., Copenhagen 8: 300-316.

EDWARDS, T. I. 1932. Temperature relations of seed germination. Quart. Rev. Biol. 7: 428-443.

EVANARI, M. 1955. "Seed germination" in radiation biology. McGraw-Hill Book Co., N. Y., 1955, pp. 519-549.

KAHN, A., J. A. Goss, and D. E. SMITH. 1956. Light and chemical effects on lettuce seed germination. Plant Physiol. Supplement 31: XXXVII.

LONA, F. 1956. L'induzione fotoperiodica di foglie staccate. Ateneo Parmense 27: 641-644.

ROHMEDER, E. 1938. Neuzeitliche Geräte und Arbeitsverfahren bei der Prüfung des Forstsaatgutes. Forstwiss. 60: 218-231.

STEARNS, F., and J. S. OLSON. 1958. Interactions of photoperiod and temperature affecting seed germination in *Tsuga canadensis*. Amer. J. Bot. 45: 53-58.

STOWE, B. B., and T. YAMAKI. 1957. The history and physiological action of the gibberellins. Ann. Rev. Plant Physiol. 8: 181-216.

TOOLE, E. H., S. B. HENDRICKS, H. A. BORTHWICK, and V. K. TOOLE. 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7: 299-324.

————, V. K. TOOLE, H. A. BORTHWICK, and S. B. HENDRICKS. 1955. Photocontrol of Lepidium seed germination. Plant Physiol. 30: 15-21, 473-478.

VAARTAJA, O. 1956. Photoperiodic response in germination of seed of certain trees. Canad. J. Bot. 34: 377-388.

WAREING, P. F. 1953. Growth studies in woody species. V. Photoperiodism in dormant buds of *Fagus sylvatica* L. Physiol. Plant., Copenhagen 6: 692-706.

Perennial Infection of Ponderosa Pine by Elytroderma deformans

BY
LEWIS F. ROTH

SYMPTOMS OF INFECTION of ponderosa pine foliage by the needle blight fungus *Elytroderma deformans* (Weir) Darker are most apparent in mid-spring. At this time infected needles of the preceding season's shoots stand out in striking red-brown contrast against the normal green foliage. As the season advances discolored foliage fades to less conspicuous brown and is partly hidden by the current season's growth of green needles. Later in the summer the fungus fruits on the faded needles, after which they fall, leaving the affected branches with a more or less healthy appearance except for deficient foliage.

Symptoms may vary from tree to tree, ranging from a general blighting throughout the crown to discoloration and malformation of a single branchlet. Except during rare periods of peak disease development general blighting is not common. Patches of blighted foliage of various sizes scattered through the crowns are the most conspicuous symptom. Branch development in the infected patches may remain normal or, in trees of good vigor the branches may proliferate and thicken to form witches-brooms which vary in density and size. The upper part of the crown of saplings and large seedlings may become broom-like. Young seedlings are rarely infected. During the early years of infection individual trees usually have more healthy than diseased branchlets.

On the Deschutes Plateau in central Oregon where the field studies were made, needle blight occurs in rather distinct areas involving only a few trees or as many as occupy several acres. These patches are hereafter referred to as infection centers. The centers appear to be of rather recent origin in the Deschutes region where the disease is not yet seriously damaging merchantable trees.

Increases in blighted branchlets from year to year occur close to old infections and appear to arise at a higher rate on trees already infected than on healthy trees. This relationship is true even though branchlets of healthy trees are often in actual contact with diseased branchlets. This behavior suggests the existence of a perennial, somewhat systemic, mycelium.

J. R. Weir (1916) was the first to report systemic infection by *Elytroderma*.

Technical paper No. 1190, Oregon Agricultural Experiment Station. Work here reported is part of a cooperative project of Oregon State College, the Pacific Northwest Forest and Range Experiment Station and the Oregon State Board of Forestry. Support is provided in part from the severance tax fund by the State Board of Forestry through its Protection and Conservation Committee.

The author is Professor of Botany and Plant Pathology at Oregon State College, Corvallis, and Collaborator, U. S. Forest Service, Division of Forest Disease Research.

However, he presented no evidence in support of his conclusions. Later, Lightle (1954), after a comprehensive anatomical study, concluded there was no demonstrable fungus in the twigs or vascular cylinder of the needles. Mycelium was limited to the mesophyll chiefly near the points of fruit body formation. Lightle observed a "distinctive" modification of the phloem of infected needles, which he interpreted as a change in the phloem cells rather than as mycelium.

While Lightle was unable to establish the presence of the fungus, the existence of a perennial infection has appeared essential to adequately explain observed symptoms. Accordingly, investigation of the matter was undertaken experimentally. Field studies were conducted at the Pringle Falls Experimental Forest near Bend, Oregon. Infection centers on Pringle Butte and Round Mountain, 2 and 7 miles distant from the station, provided necessary disease materials.

Studies in Thickets of Suppressed Pine Saplings

The characteristics of normal disease spread in understory thickets of pine saplings throw some light on the question of perennial infection. One thicket studied is located a few chains behind the advancing margin of a new infection center. The level of infection is still moderately low (40 percent of the trees and 14 percent of the branches on all trees being infected in 1955) and branchlet mortality is just beginning. Records of blighted and healthy branchlets which were recorded for the 70 ponderosa pine saplings on an area of approximately 1/100-acre are summarized in Table 1.

The table shows a low rate of annual increase in infected trees during the years of record but in the previously infected trees a comparatively high increase in infected branchlets. The increase in branchlet infections in 1957 among the previously diseased trees (21 percent) as compared with the previously healthy trees (0.95 percent) is significant. In view of the highly random scattering of the diseased branches among the healthy branches, this difference cannot be attributed to chance infection by airborne spores.

Since it is not statistically possible from the data at hand to describe the dispersion of diseased branches among the healthy ones, the observed random distribution may be questioned. However, even if the observed random occurrence of infection

TABLE 1. Characteristics of normal blight increase among the trees of an 8 x 60 foot thicket of suppressed pine saplings and among the branches of the infected trees.

Year	Blight by trees				Blight by branches							
					On trees previously infected				On trees not previously infected			
	Total trees	Blighted trees	Annual increase		Total branches	Blighted branches	Annual net change		Total branches	Blighted branches	Annual net change	
	No.	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*%*
1955	70	28	--	--	369	110	--	--	419	--	--	--
1956	70	29[1]	1	1.4	374	108[2]	−2	−0.8	420	2	2	0.5
1957	70	33[1]	4	5.7	435	164[2]	56	21.0[3]	450	6	4	0.95

[1]Respectively 28 from previous season plus three newly infected less two recovered, and 29 plus four newly infected less zero recovered.

[2]Respectively 110 from previous season plus 17 new less 19 recovered, and 108 plus 56 new less zero recovered.

[3]Twenty-one percent of the total healthy branchlets in 1956 on the diseased trees.

TABLE 2. The effect of contact or proximity of blighted and healthy branchlets on infection of the healthy branchlets after a two-year interval.

Treatment	Total branchlets treated	Branchlets found and recorded	Source branchlets continuing diseased		Receptor branchlets becoming infected	
	No.	*No.*	*No.*	*Percent*	*No.*	*Percent*
Diseased against healthy on diseased tree	20	15[1]	15	100	3	20
Diseased against healthy on healthy tree	20	18	17[2]	95	2	11
Total	40	33	32	97	5	16

[1]Several treated branches were browsed by deer or were otherwise mechanically destroyed.
[2]There was one instance of recovery of the disease source branch.

were not true it would make little difference because branch distribution and proximity to fungus fruiting bodies within an infection center has little effect on the frequency of infection by ascospores. This is shown by the following test.

Healthy branchlets were bound to blighted branchlets so that needles of the two intermingled and ascocarps of the fungus were distributed among the healthy needles. Twenty diseased shoots were bound to healthy shoots of healthy trees, and an equal number of diseased shoots were bound to healthy shoots on diseased trees. In the latter case the branches bearing the bound shoots arose at least one whorl apart. Bound shoots were not excised. Examinations were made after one and two years to allow for a possible two-year incubation period. Infection frequencies found in 1957 after the second season appear in Table 2.

Infected shoots remained infected but failed to transmit the disease to the uninfected receptors at a high rate. In only 5 instances out of 33 were the receptor shoots infected. In the two of these occurring on healthy trees receptor infection was coincident to the general infection of these trees and is considered as not related to the experimental treatment. The 16 percent increase of infected receptor branchlets is not significant because the general increase in disease severity among other trees in the area where the test was made was 12 percent for the same two years.

The foregoing observations suggest that numerous infections of new trees in the understory occur at infrequent and unpredictable intervals. Among the trees already infected the disease perpetuates itself or there is an annual increase of newly infected branchlets that appears to be largely independent of infection by ascospores. This persistence of the fungus or increase in infection, whichever the case may be, is further explained by the following studies.

Studies with Transplanted Pine Saplings

In order to understand infection trends better, the occurrence of new infections was studied in three related experiments, one begun in 1955, one in 1956 and one in 1957. Comparisons of infection frequency in the second and third of these were made in part under conditions allowing for infection by spores and conditions not allowing such infections. Seasonal disease development in previously infected branchlets also was followed in all experiments. Comparisons of blight among branchlets were possible because this disease shows symptoms

on most or all of the year-old needles of an infected branchlet or on none, a distinctive feature for a "foliage disease" and one which in itself suggests systemic infection.

Observations on trees not subject to infection from spores were made by removing the test trees from the infection center before spore discharge to an area where the disease does not occur. Spore discharge is conditioned by fall rains which in the Deschutes region may begin in September. Occasionally late August convective showers will initiate sporulation.

Since needle blight does not infect young seedlings, older trees are required for study. Well suited individuals 30 to 50 years old occur among the three-to five-foot suppressed trees of the understory of mature pine stands. These trees are readily transplanted to five-gallon buckets during late summer, thus enabling their removal from the blight center as desired (Fig. 1A).

To provide a basis for comparison of blight development in the presence and absence of spores, equal groups of experimental trees were treated as follows:

1. *Healthy trees, no treatment.* About ¼ of the foliage was pruned off to reduce the risk of transplant failure.

2. *Diseased trees, symptomatic branches pruned.* All symptomatic branches were pruned against the main stem to remove infection. This pruning left the trees healthy in appearance and enhanced transplant survival.

3. *Diseased trees, symptomatic needles clipped.* All symptomatic needles were removed from infected shoots and destroyed. This treatment eliminated the inoculum source and probably also aided transplanting.

4. *Diseased trees.* In first experiments all healthy branches were removed from this group of trees. Tree mortality became so great that in the third experiment to avoid tree loss some healthy branches were left on each diseased tree. The total crown was reduced approximately ¼ to facilitate transplanting by pruning equal numbers of blighted and healthy branches.

Trees of each treatment were divided into three lots and were distributed as follows: (1) Lot one remained within the infection center where presumably many spores lodged on the foliage and where climate, *ipso facto*, was favorable for infection and disease development (Fig. 1B); (2) lot two, depending on origin whether at Pringle Butte or Round Mountain, was moved three to six miles to the Pringle Falls Experimental Forest headquarters where elevation, climate and cover conditions approximated those of the infection centers but where trees were subject to minimal new spore inoculum if any; (3) lot three was moved to Corvallis, Oregon, where the trees were subjected to low elevation, different climate and the absence of spores (Fig. 1A).

In order to provide a basis for comparison of blight development in the presence of ascospores with development in their absence, half of the trees of lots two and three were placed at the above stated locations immediately after treatment and before spore discharge; thus they had no opportunity to become infected by ascospores. Trees of the remaining half of these lots were removed from the infection centers only after spore discharge was nearly complete. All trees of lot one remaining within the infection center ultimately were subjected to natural ascosporic inoculum. At locations outside the infection center diseased trees were isolated from healthy and treated trees to avoid cross contamination by spores.

In the first experiment, which was designed primarily to determine the feasibility of working with transplanted saplings and of studying pine needle blight outside the pine forest type, 11 blighted and 11 healthy trees were transplanted in August 1955 and moved to Corvallis. Twenty-one of the 22 trees survived and blight symptoms developed normally at least through the first year. Records were kept for four seasons. The developments appear to justify presentation of 3 years' observations of the 11 blighted trees on an individual tree basis

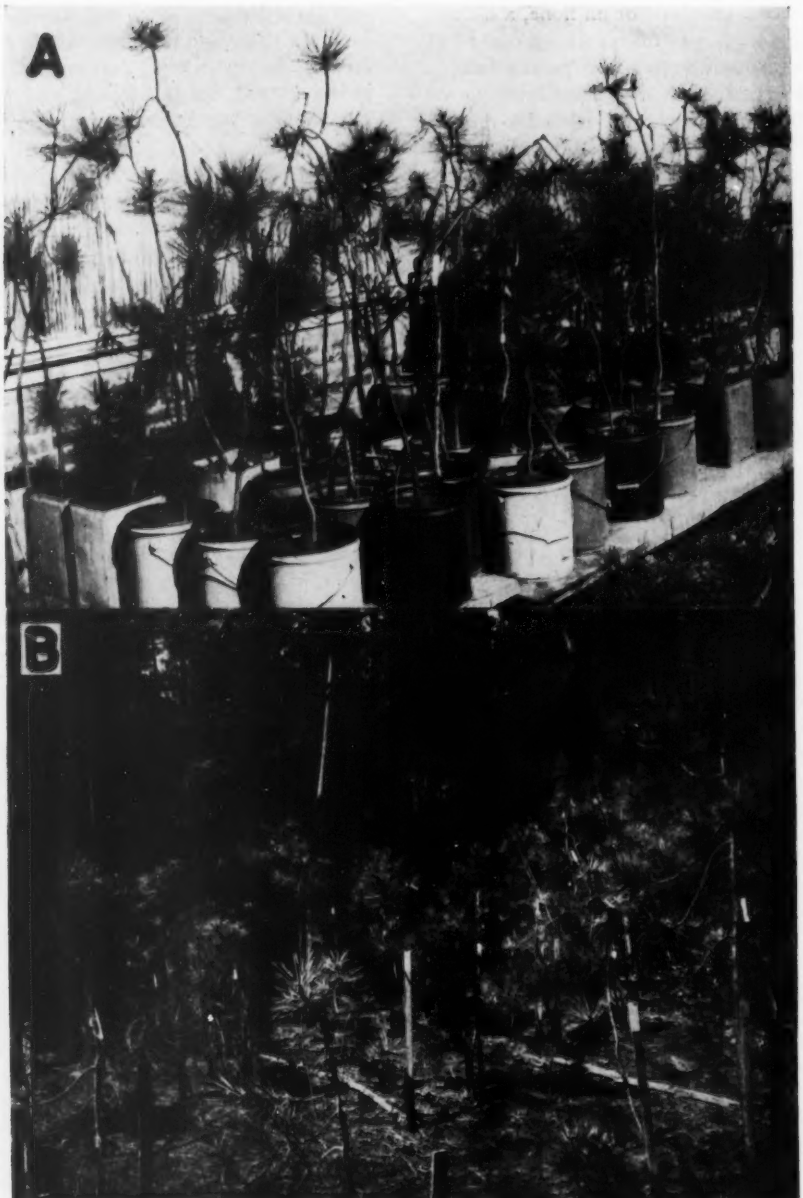

FIGURE 1. *Suppressed pine saplings having four conditions of infection described in the* **text.** ***A.*** *Trees transplanted to Corvallis. B. Marked experimental trees remaining in an infection* **center.**

in Table 3.

While most severely blighted trees succumb sooner or later (numbers 2, 6, 7, and 8), the main tendency of the trees is toward recovery. Recovery comes about through death of the very weak branches and through actual recovery of the less weakened, e.g., trees 3, 5, and 11. Trees 1, 4, 9, and 10 recovered with no branchlet mortality. Total blight in all trees expressed as percent of total branchlets decreased from 63 to 21 to 1 percent and in 1958 the seven trees remaining were disease free. Mortality for the three seasons shown respectively was 4, 29, and 12 percent. Tree and branchlet mortality resulted in a decline of living shoots from 68 in 1955 to 46 in 1956. By 1957, however, new growth began to offset the losses. In 1957 there were 51 living shoots and in 1958 there were 99.

With the exception of 4 new infections, one on blighted tree number 9 in 1956 and 3 on healthy tree number 10 in 1957, changes in observable disease status were limited to branches already symptomatic at the time of transplanting.

The fast rate of disease decline may be explained by assuming that the Corvallis climate is unsuitable for blight, and that symptoms appearing during 1956 and subsequent years resulted from systemic infection established prior to 1956.

Origin of the four "new infections" on trees at Corvallis is somewhat more difficult to explain. The newly blighted branchlet on tree number 9 was, in 1955, one of two remaining healthy shoots on a six-branch tree. If the existence of a limited systemic mycelium is allowed the infection is not surprising. In view of less favorable climate and of minimal inoculum by 1957 among the trees at Corvallis, origin of blight on three of the four branchlets of a single "healthy" tree is subject to conjecture. The possibility of latent infection deserves some consideration.

The above observations provide leads for further study. If blight symptoms were to develop on the green foliage of trees not

TABLE 3. Progress of needle blight among 11 blighted ponderosa pine saplings moved to Corvallis, Oregon.

Tree No.	1955			1956			1957		
	Healthy	Blighted	Killed	Healthy	Blighted	Killed	Healthy	Blighted	Killed
	Number of branchlets								
1	5	2	0	7	0	0	9	0	0
2	0	10	0	0	2	8	--	--	2[1]
3	1	5	0	1	1	3	2	0	0
4	1	3	0	3	1	0	8	0	0
5	5	4	0	6	3	1	15	0	0
6	1	4	0	--	--	5[1]	--	--	--
7	0	6	0	3	2	1	--	--	5[1]
8	0	3	3[1]	--	--	--	--	--	--
9	2	4	0	4	2[2]	0	6	0	0
10	5	2	0	5	2	0	7	0	0
11	3	2	0	3	1	1	3	1	0
All trees	23	45	3	32	14	19	50	1	7
Percent	32	63	4	49	21	29	86	1	12

[1]Designated trees all died the year indicated.

[2]One of these two had not been blighted in 1955. Three branchlets recovered and there was one "new infection."

TABLE 4. Incidence of needle blight in 1957 among 79 ponderosa pine saplings treated in 1956 to represent four blight conditions. Trees were in part incubated at three locations.

Treatment	Infected Branches							
	Round Mt.[1]		Pringle Falls		Corvallis		All Locations	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Healthy trees	10 (98)[2]	10	0 (31)	0	0 (56)	0	10 (185)	5
Disease pruned "healthy"	9 (50)	18	1 (33)	3	4 (44)	10	14 (127)	11
Disease clipped "healthy"	43 (45)	95	36 (36)	100	29 (29)	100	108 (110)	98
100 percent diseased	78 (78)	100	27 (27)	100	19 (19)	100	124 (124)	100
All treatments	140 (271)	52	64 (127)	50	52 (148)	35	256 (146)	47
Killed, all treatments	0 (140)	0	34 (64)	53	26 (52)	50		

[1]Trees remaining in infection centers and therefore all subject to ascospore infection.

[2]Numbers in parentheses represent total branchlets on the trees except for the "killed" line. In this case they represent total blighted shoots. They are the basis for percent computations in all cases.

exposed to spores the presence of perennial infection would be demonstrated.

The 1956 and 1957 trials, as previously described, included three and six trees respectively in each treatment, location, and time of removal from the infection center, i.e., before or after ascospore discharge. Since in tabulating the results no significant differences were found in the amount of blight between trees removed from the infection center before and after spore discharge the results were combined. Data for treatments and locations shown in Tables 4 and 5, therefore, are based on six and 12 trees respectively for the two years. A single exception exists in the use of nine trees each in the healthy and diseased categories at Round Mountain in 1956.

Branchlets of the diseased trees freed of the chance of ascosporic infection by removal of all needles bearing fruiting bodies subsequently showed foliar symptoms of infection irrespective of geographic location: 98 percent in 1957 and 100 percent in 1958. This condition is not noticeably different from that of the diseased untreated trees in which 100 percent branchlet "reinfection" occurred during both years.

All healthy trees outside the infection centers remained healthy and only one of 21 within a center became infected. This one tree with 10 of 12 branchlets blighted had symptoms more like a general systemic infection than a local one and thus probably had a latent infection contracted during a previous season.

Trees having all blighted branches pruned off flush with the main stem appeared like the healthy trees and might have been expected to react the same. This did not occur, however. During both seasons in all locations some of these trees—11 percent in 1957 and six percent in 1958—became symptomatic. Blight was no greater among pruned trees remaining in the infection center than among those removed to spore-free areas — eight percent at Round Mountain, ten at Corvallis.

Discussion

The function of the "conidia" of *Elytroderma* is unknown. In interpreting the results of these experiments it is considered that the "conidia" are not functional in in-

fection but probably are spermatial in nature.

In the absence of ascosporic inoculum the new foliage of previously blighted pine branchlets each year becomes symptomatic and bears the fruiting bodies of *Elytroderma*. As long as environment is favorable this process continues until death of the branchlet. Perennial growth within the twigs of a limited systemic fungus infection appears to be the only satisfactory explanation of these events.

During the three years of these studies ascospores played little part in the blight life cycle. Their limited function is seen in the rare occurrence of infection of healthy trees, even in natural infection centers where branches of the healthy trees were intermixed with the foliage of blighted trees on which the fungus was fruiting. Blighted and healthy foliage bound together similarly failed to result in significant infection of the branches of healthy trees.

While there was very little infection of the branchlets of healthy trees by ascospores there was a significant increase in blighted branchlets on the diseased trees. Such increases were especially notable in the Round Mountain infection center where, in 1958, 33 new branchlet infections—19 percent—occurred among the 24 diseased trees. New infections regularly appear on branchlets adjacent to old infections which in the absence of effective ascospores indicates their systemic origin. Probably fungus invasion and new shoot growth occur concurrently during the season preceding the red foliar symptoms. Symptoms appear very early in the spring up to which time there is very little mild weather and probably little opportunity for fungus growth. Systemic development appears to be not limited to succulent tissues of the current season. Areas of crown infection become progressively larger by involving shoots adjacent to those already diseased. Presumably this occurs by systemic means requiring fungus growth through stems having mature tissues.

The rare occurrence of new infections on previously healthy trees in the infection centers suggests that ascosporic infection is

TABLE 5. Incidence of needle blight in 1958 among 144 ponderosa pine saplings treated in 1957 to represent four blight conditions. Trees were in part incubated at three locations.

Treatment	Infected branches							
	Round Mt.[1]		Pringle Falls		Corvallis		All Locations	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Healthy trees	0 (106)[2]	0	0 (78)	0	0 (75)	0	0 (259)	0
Diseased pruned "healthy"	7 (146)	5	3 (69)	4	8 (78)	10	18 (293)	6
Diseased clipped "healthy"	114 (93)	122[3]	55 (53)	101	33 (36)	92	202 (182)	111
Disease untreated	193 (181)	106	26 (26)	100	54 (56)	97	272 (263)	102
All treatments	314 (526)	60	84 (226)	37	95 (245)	39	493 (997)	49
Killed, all treatments	29 (314)	9	35 (84)	35	6 (95)	6		

[1]Trees remaining in infection center and therefore all subject to ascospore infection.

[2]Figures in parentheses represent total branchlets on the trees except for the two groups: "clipped healthy" and "diseased untreated." In these latter cases the figures represent only the total diseased branchlets in 1957 at time of treatment. In all cases the figures in parentheses are the basis for percent computations.

[3]New infections appearing in 1958 on branchlets rated as healthy in 1957 in some cases caused the percent of blight to exceed 100.

very closely regulated and does not occur abundantly every season the fungus sporulates. Similarly the systemic phase of development is somewhat dependent on environment. The years 1956 and 1957 differed greatly in this respect (Table 1) in the same locality. For example, branchlet recovery was common during 1956 but did not occur in 1957. Differences of locality also exert an influence. Infection centers on the Deschutes Plateau, while widely scattered, probably occur in areas well suited to disease development. In these areas there has been a progressive increase in systemic spread, even though ascosporic spread has been quite limited during the period of observation. Larger portions of the lower crowns of overstory trees become involved and the crowns of saplings in the understory are slowly but progressively blighted until death of the trees results.

Outside the infection centers but still in the pine area, disease advance occurs at a very slow rate or not at all, and outside the pine region under different climatic conditions there is definite progress toward recovery from systemic infection. In one instance diseased saplings taken to Corvallis improved so that after 3 years all were healthy.

These findings conform with field observations of recent years (Childs, 1955; Hunt and Childs, 1957) and suggest that the recent blight epidemic of the Intermountain Region arose during one or several years especially favorable for ascosporic infections. The years immediately following were characterized by a high level of tree mortality resulting in serious losses and extensive salvage logging.[1] The disease is currently maintaining a high chronic level by the systemic spread of existing infections and by limited increase by ascospores. It may not be expected to decline rapidly from the present level until some event occurs that is adverse to development of the perennial phase.

From the standpoint of field application this situation suggests that little may be expected from control efforts by traditional protective spraying methods.

The time and place of infection by *Elytroderma* is unknown. While occasionally blight lesions of limited size occur on scattered needles, these seem more often associated with the decline of infection in a branchlet than with its origin. The symptoms usually thought of as representing "new infections" appear simultaneously and more or less completely on the long normal needles of a previously "healthy" branchlet. The interval between initial entrance of the host by the germ tube and the time of appearance of these general symptoms also is not known. If infection occurs through a needle, time sufficient to grow through the needle into the bud appears to be the minimum for fungus distribution to the critical tissues. Red foliage symptoms, however, will not appear until a year after the bud breaks. Observations suggest that the time required for symptom development may vary widely and that there may be a period of latent fungus development.

[1]From the records of Forest Supervisor Vondis Miller, it is noted that on the Ochoco National Forest alone, in central Oregon, sales of needle blight timber between August 1947 and June 1951 totaled 139,295 M bd. ft.

Summary

Partially blighted ponderosa pine saplings growing in blighted forest areas were compared as to disease development with similar trees transplanted to disease-free areas. Trees in three disease conditions were included along with healthy checks: (1) diseased, unpruned, (2) diseased, with all blighted branches pruned off, (3) diseased, with all blighted needles removed. Irrespective of treatment, foliage of once blighted branches became symptomatic the year following treatment. Branchlets of some of the pruned trees became symptomatic in the first season following treatment while healthy trees remained healthy. There were no differences in performance the first season between trees in the

blighted forest and those in disease-free locations. In subsequent seasons the latter trees recovered. These results lead to the conclusion that branchlets are systemically infected, that the infection can be perennial and may extend back into woody branches several years old. Ascospores contributed little to the level of infection prevailing during the years of study.

Literature Cited

CHILDS, T. W. 1955. Needle blight of ponderosa pine. Pacific Northwest Forest and Range Expt. Sta. Research Note 114. 5 pp.

HUNT, JOHN and T. W. CHILDS. 1957. Ponderosa pine needle blight in eastern Oregon during 1955 and 1956. Pacific Northwest Forest and Range Expt. Sta. Research Note 147. 9 pp.

LIGHTLE, PAUL C. 1954. The pathology of *Elytroderma deformans* on ponderosa pine. Phytopathology 44: 557-69.

WEIR, J. R. 1916. *Hypoderma deformans* an undescribed needle fungus of western yellow pine. J. Agric. Res. 6: 277-288.

Data for Planning Woodland Opportunities on West Tennessee Farms

By Solon L. Barraclough and Alfred Pleasanton, Bulletin 276, University of Tennessee Agricultural Experiment Station in cooperation with the Southern Forest Experiment Station, November 1957. 63 pp., illus.

Review by E. M. Gould, Jr.,
Lecturer on Economics, Harvard Forest, Petersham, Mass.

Of late years we have heard a great deal about the industrial revolution now going on in the South. Most of this talk is in such general terms as new pulp plants, more active markets, and their stimulating effect on the economy. All too little has been published that will help individual landowners and operators to assess the impact that these developments may have on their own management opportunities. For the last few years, however, a well-conceived research program has been under way at the Ames Plantation in Grand Junction, Tennessee. In cooperation with the Jula Ames Will Trustees, the University of Tennessee has set out "to test new and promising farming systems that include forestry." These practical, on-the-ground tests and the data derived from this pioneering effort are helping individual landowners to find out what modern technology and the new economic climate can mean to their own operations.

This bulletin contains the results of the first three years of forest research and testing at the Ames Plantation. The first section summarizes the work-performance data that have been carefully collected in the course of planting over a million trees, releasing pine from hardwood suppression on about 330 acres, improvement

girdling on about 120 acres, harvesting 550 units of peeled hardwood pulp and over a million feet of sawlogs. This section shows not only the average and the range of input-output relationships experienced, but also includes a good discussion of the major factors that affect work performance on each job in a way that will help a forest manager to select planning data geared to his own operation.

One of the most interesting observations concerns worker morale and fatigue. After two years of working a 50-hour week at 45 cents an hour the woods crew was shifted to a 60-hour week and paid 85 dollars a month. The average work required to fell and buck a thousand board feet rose from 6.6 man-hours to 9.6 hours, an increase of about 45 percent in labor inputs and about 10 percent in cash costs.

The second section concerns management-yield relationships, and is necessarily based on a projection of the long-run yields likely to accrue from specific management programs carried out on typical forest stands at the Ames Plantation. Nine tables show the yields expected, by decades, until stable levels are reached. Two sets of figures show the probable cut under "intensive" or under "extensive" management, together with the estimated labor input for "management" and "harvesting." A full-page picture gives a good impression of each of the stand types discussed. One could wish, however, that a little more description of the management program proposed for each stand condition had been included as a footnote to each table.

After a discussion of price trends and relationships, the final section demonstrates how the work-performance, management-yield, and price data can be used to lay out alternative programs of resource use for a specific operating unit. The tenant-operated farm discussed has major opportunities for investment in both agriculture and forestry, and the budgets of likely alternatives drastically reduce the area of guess-work in connection with the financial problems that the tenant and landlord must weigh in choosing a plan of action.

This bulletin should be especially useful to foresters in the South, and in addition it lays out a format for data than can be applied anywhere. More universal collection of comparable data in the right form could quickly supply a great deal of the information needed for more penetrating analyses of forest management opportunities. This report shows that rapid progress can be made, even as a by-product of regular forest operations, and one hopes that the next three years will be equally productive at the Ames Foundation.

The Biology and Ecology of the Balsam Gall Midge in Wisconsin

BY
RONALD L. GIESE
DANIEL M. BENJAMIN

IN THIS ERA of keen competition in the Christmas tree industry, insect damaged trees are difficult to market. The balsam gall midge, *Dasyneura balsamicola* (Lintner), long referred to as *Itonida balsamicola* (Lintner), infests elongating needles of balsam fir, *Abies balsamea* (Linn.) Mill., and causes the formation of subglobular swellings (Fig. 1). Though this deformity is not particularly objectionable, the problem becomes acute when the needles drop after the tree reaches the consumer.

FIGURE 1. *Galls caused by the balsam gall midge.*

Nearly 7,000,000 balsam fir trees are purchased annually in the United States as Christmas ornamentals. Tree farms in Wisconsin contribute 14.3 per cent of these. Balsam fir is ideally suited for Yuletide purposes because of its long needle-holding capacity (with or without water), firmness of branches, attractive form, fragrance and resistance to fire. Undoubtedly, its popularity will continue and in many northern cities an increase in demand is expected as the market turns from the multitude of exotic species to this traditional

---

Excerpts from a thesis submitted in partial fulfillment for the requirements for the M.S. degree in Entomology. The project was conducted under a grant from the Division of Forestry, Wisconsin Conservation Department. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

The authors are Research Assistant and Associate Professor of Entomology, respectively, University of Wisconsin, Madison.

favorite.

To prevent widespread economic loss caused by the midge, knowledge of its bionomics must be acquired. Through the application of such information future epidemics can be handled intelligently.

Gall Midge Outbreaks and Distribution

Attention was first called to the midge in 1886 during an outbreak in New York (Lintner, 1888). The insect attained such notoriety that Lintner was prompted to describe this new pest and commented that ". . . at least three-fourths of the leaves of the tips of this season's growth bore the galls."

The midge has been reported from most parts of the range of balsam fir (Fig. 2). In the United States outbreaks were reported in Maine in 1957 (J. B. Dimond[1]), Massachusetts in 1938 (W. D. Becker[1]), Michigan in 1956, New Hampshire in 1939 (J. G. Conklin[1]), and Wisconsin in 1939, 1956, and 1957.

In Canada knowledge of the extreme northern limit is restricted by the lack of roads and surveys. However, surveys indicate its presence as far north as the Otish Mountains in Quebec, near the 53rd parallel.[2] Damage has been found throughout Quebec (Daviault, 1947; Daviault and Gobeil, 1942; Lambert, 1941, 1942, 1949), Nova Scotia (Balch, 1939, 1940), New Brunswick and Ontario (Reeks *et al.*, 1947a, 1947b; Jarvis, 1908; Forbes,

[1]From correspondence in the files of the authors.

[2]The authors are indebted to W. L. Sippell, R. Béique, and R. S. Forbes for distribution data from Canada.

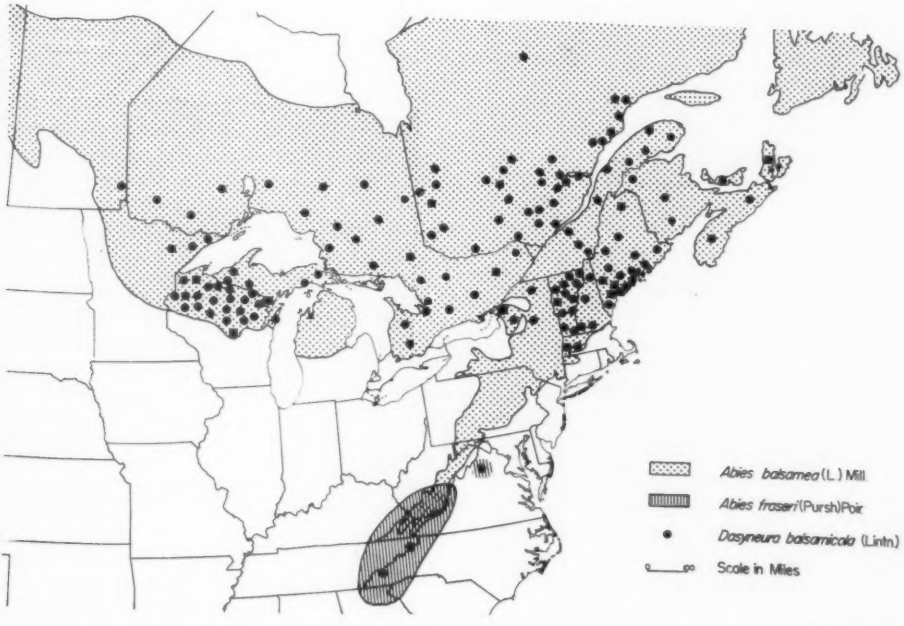

FIGURE 2. *Distribution of balsam fir, Fraser fir and the balsam gall midge in North America.*

1957), Prince Edward Island and in the Whiteshell Forest Reserve in Manitoba. W. A. Reeks[1] reported typical balsam needle galls in northern Ontario, Manitoba and Saskatchewan, but was not certain of the identity of the causal agent. Barnes (1951), however, stated that *D. balsamicola* is the only species of Itonididae attacking balsam fir needles.

The midge has been recorded on Fraser fir (*Abies fraseri* (Pursh) Poir.) in North Carolina (Lintner, 1890; Wilford, 1929), and in Virginia (C. L. Norris[1]).

Taxonomic Position

The balsam gall midge and its gall were described by Lintner (1888) as *Cecidomyia balsamicola* from a collection of galls and a single, dead larva received in December. Lintner was unsuccessful in rearing adults, and later workers also reported the adult midge to be unknown (Aldrich, 1905; Barnes, 1951; Craighead, 1950; Felt, 1906, 1918, 1925, 1940; Leonard, 1928). According to Chamberlin (1946), the discovery of Meigen's paper of 1800 resulted in the generic name *Cecidomyia* being changed to *Itonida* and the family name from Cecidomyiidae to Itonididae. R. H. Foote of the U. S. National Museum identified balsam gall midge adults, reared by the writers, as *Dasyneura balsamicola* (Lintner), thus establishing for the first time the true taxonomic position of this insect.

Life Stages

During the field seasons of 1956 and 1957, egg and larval stages were collected at biweekly intervals in northern Wisconsin. Measurements of the general body length, head and breast plate of 100 larvae per sample were recorded from slide material mounted in de Faure's medium.

Mature larvae leaving the galled needle in late fall to overwinter in the litter were collected in conical polyethylene traps beneath infested trees. After exposure to below freezing temperatures for two months, larvae were held at 40° F. for 14 days, then placed on moist filter paper at room temperature.

Adult female. Length 3.7 mm. (including antennae .7 mm. and body with the ovipositor extended). The antennae (Fig. 3D) are dark brown, 14 segmented (plus scape and pedicel), and segments of the clavola are not separated from each other. They are twice as long as broad except for the ultimate segment which is four times as long as broad. Whorls of hairs arise from each. The palpi (Fig. 3A) are quadriarticulate with the terminal segment longer than each of the previous three.

The thoracic dorsum is brown; the legs are brown also, due to pilosity, and are 2 mm. in length. The wings (Fig. 3B) (length 1.7 mm. and width .7 mm.) are hyaline, pilose and fringed (not shown). The third vein curves slightly forward near its apex and unites with the costa before the wing tip. The median is represented by a fold, which gives the appearance of being slightly more clothed with hairs than the rest of the wing; the fifth vein is forked.

The abdomen is generally orange with tan areas on each of the tergites. Evidently, the ovipositor is capable of being withdrawn telescopically; the dorsal chitinous rods are visible on the basal segment of this structure.

Adult male. Length, 3.5 mm., including the antennae (Fig. 3C) which are 1.7 mm. in length, dark brown and 16 segmented (plus scape and pedicel). The segments are nearly spherical and separated from each other by internodes each one half the diameter of the segments. Each segment has several whorls of hairs, some up to 2½ times the length of the segment. The palpi are four segmented. The general body color is tan with a brown thoracic dorsum. Legs of the male are dark brown and 2 mm. in length. The tarsal claw (Fig. 3G) is dentate, long and sickle shaped and has the basal tooth arising at an approximate

right angle. Wings (Fig. 3E) of the male are similar to those of the female.

The abdominal tergites have darkened areas. The basal clasp segment of the genitalia (Fig. 3F) is irregularly shaped and twice as long as the terminal.

Egg. The egg is oval, whitish, translucent and about .35 mm. in length.

Larvae. Williston (1908) noted that "It is probable that the larvae of itonidids generally do not undergo molting like most dipterous larvae." This was generally accepted by others working with the order

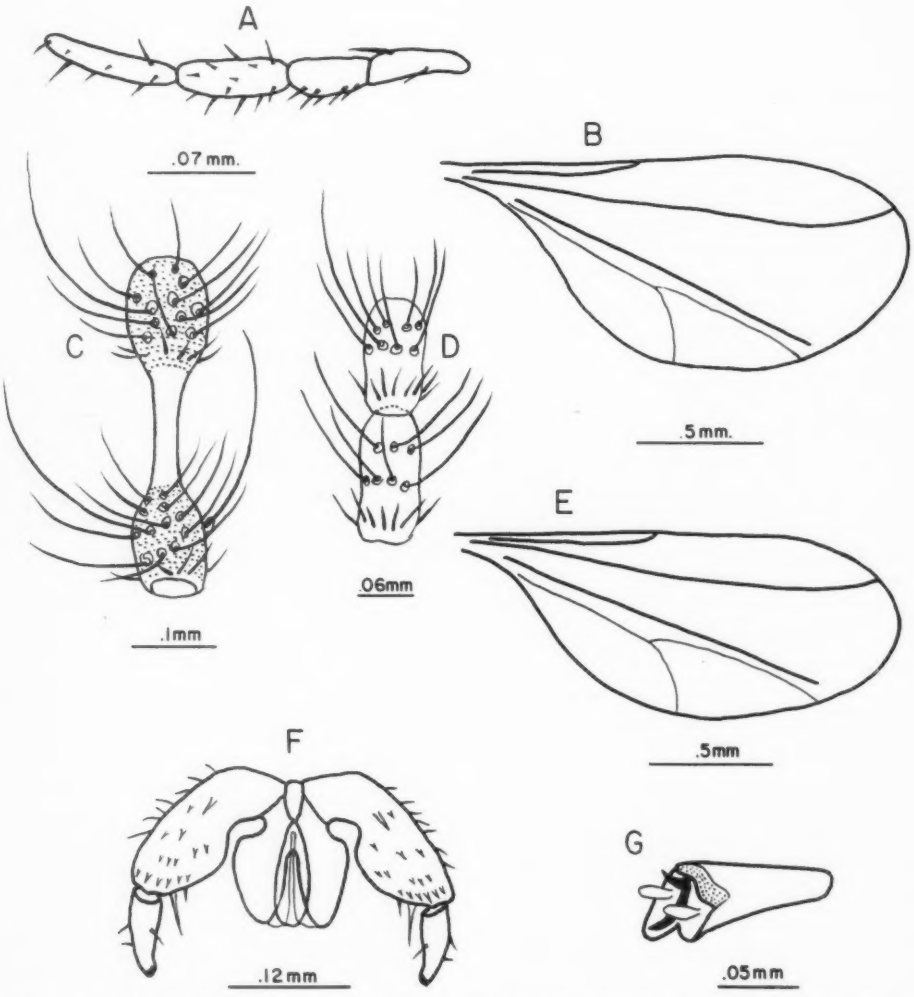

FIGURE 3. *Female: A–Quadriarticulate palpus. B–Wing. D–Fifth and sixth segments of antenna. Male: C–Fifth and Sixth segments of antenna. E–Wing. F–Genitalia. G–Hind tarsal claw.*

(Aldrich, 1905, and Curran, 1934). Foote (1956) states that little research has been done on the biology and morphology of the family because concepts of species

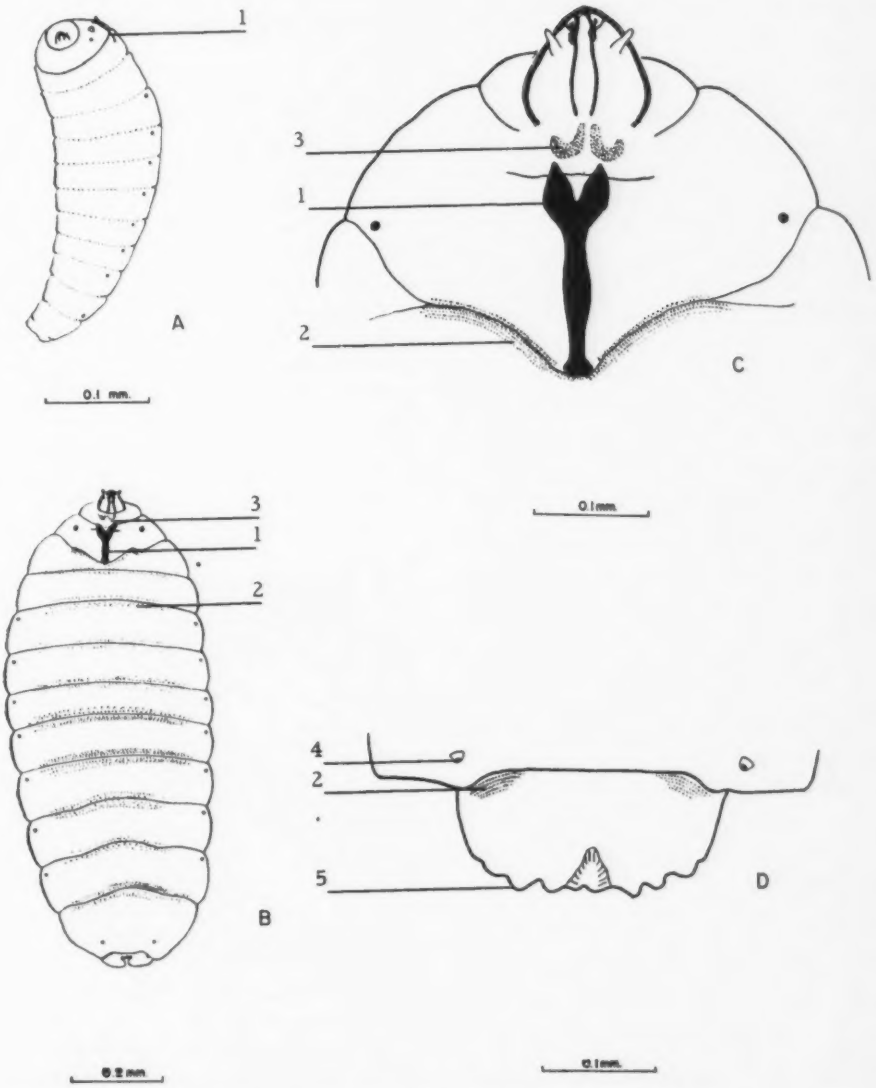

FIGURE 4. *Larvae of* D. balsamicola: *A—Lateral aspect of 1st stage larvae. B—Ventral aspect of mid-stage larva. C—Ventral anterior portion of the late-stage larva. D—Ventral posterior portion of the late-stage larva. (1—breast plate, 2—microtrichae, 3—wing shaped structure, 4—dome shaped spiracles, 5—anal papillae.)*

differentiation for the larval stages are not clear.

Measurements of the head and breast plate (Fig. 4, B1, C1) from larvae collected throughout the summer reveal three larval stages. Dome shaped spiracles (Fig. 4, D4) occur on segments 2 and 5 to 12 inclusive; semi-lunar structures, the identity and function of which are not known, occur anterior to the breast plate (Fig. 4B, C3). Minute projections, believed to be microtrichae, are concentrated at the segmental sutures (Fig. 4B, C, D2). The breast plate is furcate anteriorly.

First stage larva. The first stage larva (L1) (Fig. 4A) is whitish and translucent. The head is broad and the larva tapers gradually toward the posterior. The general body length is 0.3 mm., the head capsule 0.03 mm., and the breast plate 0.04 mm. ± .004 S. D.

Mid-stage larva. The L2 (Fig. 4B) is pale yellow at first, turning darker toward the end of the stadium. The length of the body averages 1.3 mm., ranging from 1.1 to 2.0 mm., the head capsule 0.05 mm., and the breast plate 0.08 mm. ± 0.009 S. D.

Late stage larva. Late instar larvae (L3) have a bright orange color. The length of the body averages 3.0 mm., ranging from 2.7 to 3.4 mm., the head 0.7 mm. and the breast plate 0.2 mm. ± 0.02 S. D. Möhn (1955) uses the anal papillae (Fig. 4, D5) as specific characters in separating the genera and species of itonidids.

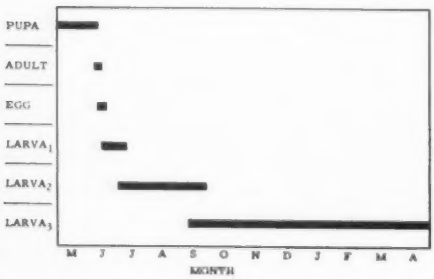

FIGURE 5. *Periodicity of the life stages of the balsam gall midge.*

Pupa. The exarate pupa is orange, 2.0 to 2.5 mm. long with unidentate cephalic horns.

Life History

The mature larva overwinters in the mineral soil beneath infested trees. In the laboratory, a frail, whitish cocoon is spun. Undoubtedly, this structure is spun in the field also, although it was not observed.

Pupation occurs in the spring (Fig. 5) and required an average of 15 days in the laboratory. The breast plate is cast off and invariably found within the cocoon. Toward the end of its development, the eyes, antennae, wings and legs become black as the pupa becomes densely covered with pile. The adult emerges through a longitudinal fracture along the thoracic dorsum.

Newly emerged adults require several hours for sclerotization and wing expansion. Longevity in the laboratory averaged one and one-half days and in several cases adults lived for two days. Adults emerged over a period of 9 days, the males emerging first. The sex ratio, based on 53 specimens, is about 1:1.

Twigs collected on May 10 in an area of heavy infestation showed no signs of hyperplasic or hypertrophic development, although elongation of twigs and needles had commenced. Galls were observed by the second week of June. Although oviposition was not observed, it apparently took place during the last three weeks of May.

The first larval stage (L1) persists for only two or three weeks. Frequently, in the early season, galls contain a larva and an egg which may not hatch for two weeks. When more than one larva per gall occurs, there is almost always a size difference. Though a single larva initiates the gall stimulus, two or more larvae are frequently found within the gall. A mean of 1.4

larvae per gall was found with a range of 1 to 6. The most frequent number was two.

Second stage larvae (L2) were observed on June 26, and continued their development until September 10, when transformation to the late stage (L3) began. The larvae continue to feed until late fall.

The mature larvae escape via the *exit orifice* (Figs. 6, 7), squeezing out of the gall at a right angle between the lips. The process requires several hours. The larvae drop and overwinter in the soil; abandonment of the galls usually occurs in mid-October.

After reaching mineral soil, the larvae enter diapause. Diapause appears to be terminated by an extended exposure to low temperatures. Larvae held at freezing temperatures for several months, then incubated at room temperature, resumed development. Larvae not exposed to freezing did not complete metamorphosis.

FIGURE 6. *The mature larva emerging from the gall.*

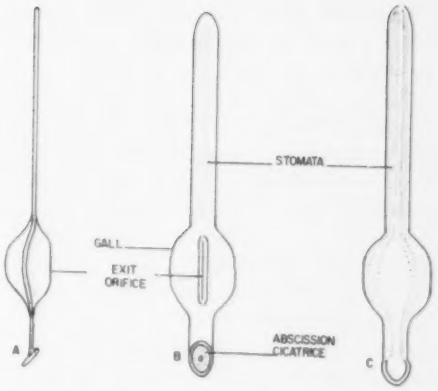

FIGURE 7. *Morphology of the gall caused by the balsam gall midge: A—lateral aspect, B—dorsal aspect, and C—ventral aspect.*

The Gall

Balsam gall midge larvae apparently are the primary stimulus for gall formation, either by mechanical irritation or by a combination of mechanical and chemical stimuli. The response of the plant tissue is reflected in leaf folding, with the epidermis lining the gall interior. Weigel and Sandford (1920) also reported this with chrysanthemum midge eggs deposited on the leaves. Plumb (1953) reported fumigation of the gallicolae of *Chermes* (*Adelges*) *abietis* L. did not prevent the completion of the gall once it had started development. Evidently, the converse is true for the balsam gall midge. On numerous occasions galls were observed that had not developed past the rudimentary stage. Two inconspicuous elevations represented the folding leaf, which would normally have given rise to the longitudinal lips encompassing the exit orifice. In every instance, the arrested growths contained dead midge larvae.

Galls of the midge are monothalamous enlargements, ellipsoidal in shape. Both hypertrophy and hyperplasia occur in the cecidium. The most prominent feature is a lip-like structure, the "*exit orifice*" (Fig.

7), formed by the upfolding of the leaf tissue. This aperture provides a means of escape for the larvae.

Cosens (1912) summarized other modifications of tissue associated with gall formation on balsam needles, and cited the average gall dimensions as length 2.5 mm. and width 1.5 mm. Under normal conditions, this is true, but several exceptions mask generarlized dimension limits. Firstly, in epidemics, multiple galling is frequent, and up to five galls may be formed on a single needle. Such malformity results in great distortion, and if the entire needle is involved in gall enclosure individual galls are difficult to separate. However, though the extremely large swelling appears as one gall, it actually is made up of a number of small galls which have identifiiable orifices. Occasionally, two chambers may have a common opening. The second exception is found commonly on trees which have had tops cut for Christmas trees with several whorls left for vegetative reproduction of a second crop. With a root system already established, the new needles grow to enormous proportions (Fig. 8). Often they are susceptible to infestation by the midge, and the resulting galls may be more than twice the size of the typical forms.

FIGURE 8. *A typical twig from a remaining whorl of a previously top-cut tree, showing abnormal increase in needle size. (Note that most of the infested needles have already fallen from the twig.)*

The single-galled needle. Typically, a single gall is formed with the exit orifice on the dorsal surface of the needle parallel to an imaginary longitudinal median line (Fig. 9, I, A). This gall, the "typical single," occurred in 82 percent of the single galled needles. The "A subtypical single" (Fig. 9, I, B) and "B subtypical single" (Fig. 9, I, C) with the exit orifice on the ventral surface, formed a second prominent group, comprising 6 and 5 percent of the single galled needles, respectively. The remaining galls were comprised of more than a dozen variations (Fig. 9, I, D and F in part).

The double-galled needle. In outbreaks, the single gall is found infrequently. The "typical double" (Fig. 9, II, A), which has both orifices on the dorsal surface, one located on the lower one-third of the needle and the other on the middle third is commonly encountered. The exit orifices parallel the long axis of the needle in a median position. The occurrence of the "typical double" represents 37 percent of the total for all double galled needles. In double-galled needles, secondary types assume a position of greater importance. The "A" and "B" subtypical double galls (Fig. 9, II, B and C), occur 17 and 14 percent

of the time, respectively. The difference is made up of numerous variations (Fig. 9, II, D and E in part).

The triple-galled needle. Three galls are most common in heavy infestations. The "typical triple" gall (Fig. 9 III, A), disrupts the pattern of "typical single" and "double" galls. Only the distal gall has the orifice on the dorsal surface. The other two galls have the aperture on the ventral surface positioned on either side of the midrib. The form with all three galls on the dorsal surface (Fig. 9 III, E) (as in the typical "singles" and "doubles") represents a minute portion of the existing triples. The "typical triple" represents 47 percent of all triple galled needles. The "A subtypical triple" (Fig. 9 III, B) and the "B subtypical triple" (Fig. 9 III, C) appear as typical doubles with an extra gall opening on the ventral surface on the left or right side. These occur in secondary prominence, the percentage being 14 and 13 for the "A" and "B" subtypes respectively. The remainder of the samples included many variations (Fig. 9 III, D and E in part).

Other multiple gall formations. Needles with four galls have typical forms nearly identical to the "typical triple" except that an extra dorsally-opened gall occurs distally (Fig. 9, IV, A). Several of these are shown (Fig. 9, IV, A and B). Five galls often occur on single needles in outbreaks (Fig. 9 V, A and B). The formational possibilities are so great and identity becomes so obscure that morphological classification was not attempted.

Analysis of Gall Distribution on Balsam

The midge is considered endemic when one to 50 needles per twig are galled and less than one percent of the twigs are infested. Seldom would more than two galls per needle be encountered.

The midge is considered epidemic when more than one percent of the current twigs have infested needles. Three to five galls per needle are common.

Within each outbreak area, a few trees generally have nearly all of the current foliage galled. The severity of infestation declines progressively to a zone of light infestation, where galls are difficult to find. Examination of leaf scars on previous years' twigs reveal earlier infestations, indicating that these heavily infested trees undoubtedly harbored gall midge larvae during the endemic period.

In the infestations studied, from one to 143 galled needles occurred on single balsam twigs containing from 40 to 250 needles. In endemic areas, single twigs harbored up to 50 galled needles, but the most common number of galls per twig was one, representing 16 percent. The number of galls per twig in the 26-50 category was quite rare, and each class represented less than one percent of the total. A reversal was evident in epidemics, and the low number of galled needles per twig became the exception. Often from 90 to 95 percent of the total needles on the twig were infested. The most frequently encountered number of galls per twig fell between 100 and 143, while the number of galled needles in the one to 25 classes was less than 5 percent.

In epidemic conditions, the greater the number of infested needles per twig, the greater the number of needles severely deformed with multiple galls. As the number of infested needles per twig increased, a highly significant correlation between the number of double- ($r = +0.924^{**}$) and triple- ($r = +0.904^{**}$) galled needles became evident.

The correlations between a multiple gall formation and the next higher level of individual needle infestation also were highly significant in the following relationships: (a) the greater the number of double-galled needles per twig, the greater was the number of triple galls occurring on the same twig ($r = +0.802^{**}$), (b) the greater the number of triple-galled needles per twig, the greater was the number of quadruple-galled needles on the same

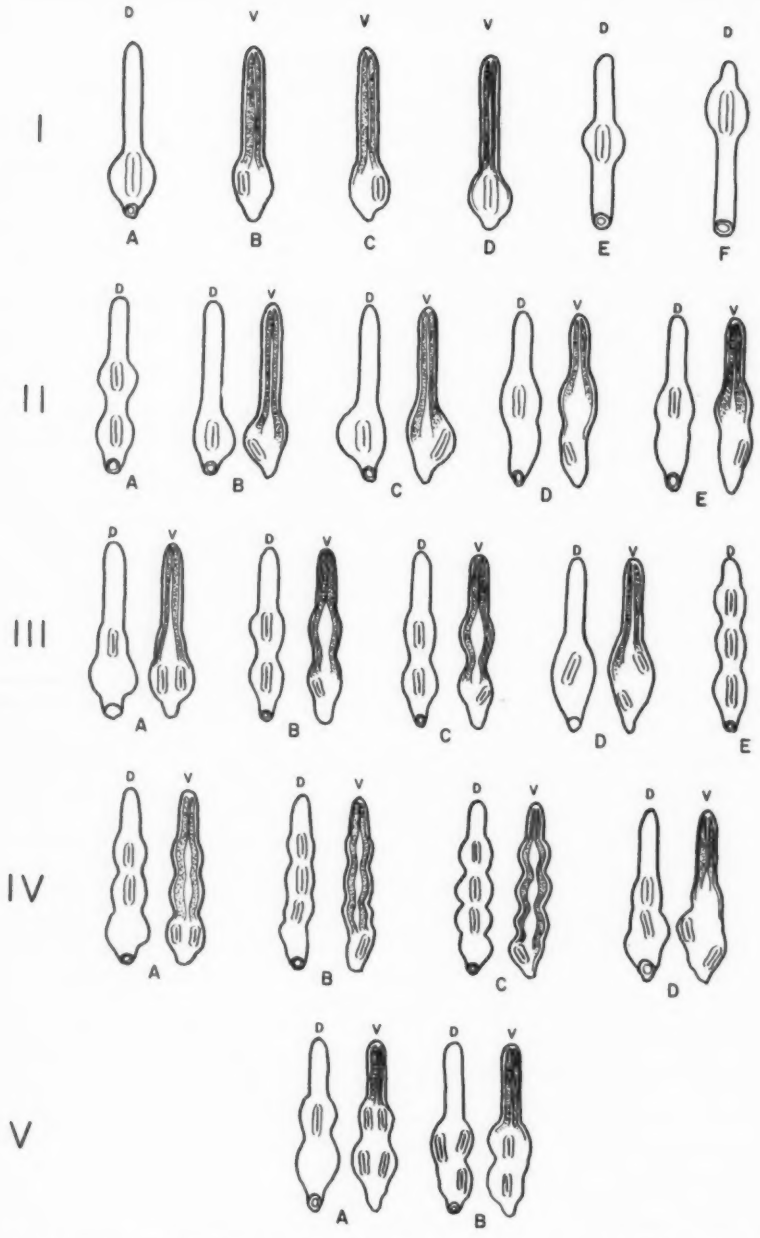

FIGURE 9. *Variations of galled needles: Row I—Single galled needles. Row II—Double galled needles. Row III—Triple galled needles. Row IV—Needles with 4 galls. Row V—Needles with 5 galls. (D—dorsal surface, V—ventral surface.)*

twig ($r = +0.870^{**}$). In endemic situations, these relationships were not demonstrable.

These relationships are basic to the design of a survey procedure. Analysis of the number of triple- or quadruple-galled needles on a twig will yield the approximate number of infested needles per twig. The infestation level may then be derived, because any number of galls per twig over 50 is a direct function of the degree of infestation.

Vertical distribution. The vertical distribution of galls is nearly uniform, with a slight excess on the upper crown on trees up to 20 ft. in height. Although galls have been found as high as 43 ft. above the ground, heavy infestations were limited to trees less than 25 ft. in height. In open growing stands, all current needles are susceptible, but with shading either by a hardwood overstory or intraspecific crowding; the lower and inner branchlet current needles are seldom attacked. In epidemics, all twigs on apical branches and the terminal shoot usually have 90 percent or more of the needles infested.

Radial distribution. Consideration will now be given to the radial distribution of infested twigs bearing galled needles and the age of wood giving rise to the primary elongation. In order to facilitate the discussion, a diagrammatic sketch of a nine-year-old branch from an epidemic area will be used (Fig. 10). A progressively declining number of twigs contain galled needles from the current elongation of the primary axis, toward the main stem of the tree. Specifically, on the primary elongation, whose date of origin was 1957, all current twigs bore infested needles. On the primary elongation originating in 1956, 1955, 1954, etc., 92, 77, and 65 percent of the current twigs bore infested needles respectively. Current growth on wood of 1950 origin bore no infested needles.

A similar relationship exists between the number of twigs bearing galled needles

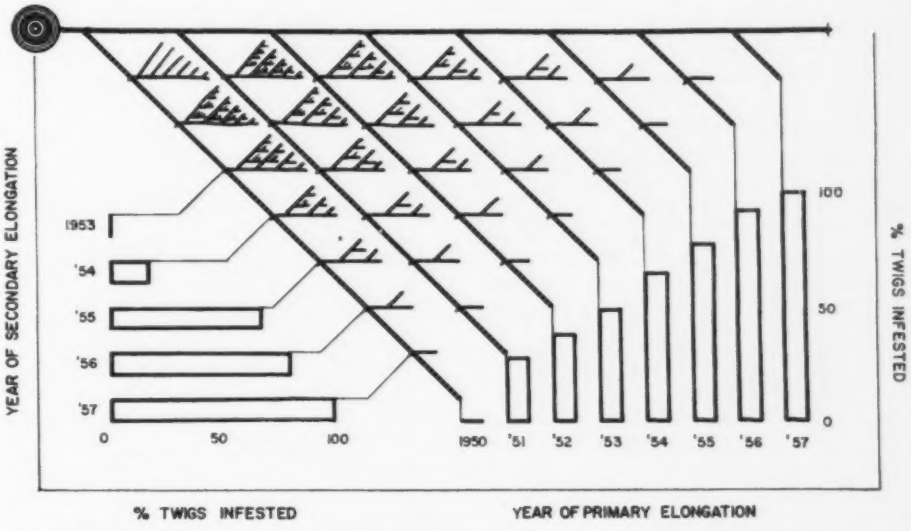

FIGURE 10. *Diagrammatic branch illustrating percent of twigs infested with balsam galls for primary and secondary elongations.*

and the year of origin of secondary elongation, e.g., secondary elongations whose date of origin was 1957, bore galled needles on 96 percent of the twigs; secondary elongation originating in 1956 had infested needles on 76 percent of the current twigs (1957) and secondary elongation of 1955 origin had 64 percent of the twigs bearing galled needles.

On current needles arising from tertiary and quaternary elongations, the distribution of galled twigs followed the same pattern demonstrated by primary and secondary elongations. This analysis enabled the formulation of accurate sampling in the horizontal and vertical universe and a prediction of subsequent needle fall.

Balsam needle abscission. Balsam fir normally retains its needles for four or more years. Infestation of needles by the midge disturbs this physiological equilibrium and infested needles fall prematurely.

At the end of the first growing season, an abscission layer forms in infested needles. By the end of September, galled needles begin dropping, and by the following spring, over 90 percent of the previously infested needles have dropped. Wind, frost, snow, rain, and the weight of the needles themselves evidently contribute to rupture of the vascular elements and the final detachment of the needles. The abscission scar is clearly defined on the twigs which harbored infested needles.

Influence of Site and Tree Vigor

Widespread areas of natural balsam fir occur under a variety of conditions throughout northeastern North America. Suspectibility to attack by the midge also varies greatly. Before recommendations for silvicultural control of the midge can be attempted, ecological factors influencing the host and its parasite throughout their respective ranges must be known. Cursory observations of some of these factors will be briefly discussed.

Ten plots in Oneida County, Wisconsin, were selected in a variety of size classes, in both the upland and lowland habitat. Five trees constituted a plot. The diameter of the stem of each tree was measured, and the height was measured with an Abney level. An increment boring and a 20 cc. soil sample were taken at each station. Soil pH was determined by use of the Beckman pH meter.

Mature balsam were often infested with the midge. However, less than one percent of the current twigs harbored galls. Heaviest infestations were found on seedlings and trees to 25 ft. in height.

Trees most subject to heavy infestation generally had a low height for their age and diameter. Diameter-height curves for these trees had low slopes ($b = +2.12$); these were significantly less than curves for noninfested and lightly infested trees ($b = +4.76$).

Exposure was of little significance and galled needles were distributed equally in all quadrants. However, stand density appeared to play some part as galled needles were most common on the margins of dense stands, while open growing forests were infested throughout.

There appears to be a definite relationship of midge infestation to the elevation of the habitat. All heavy infestations were found in lowland sites, except on one well-drained area on which forest pulled stock had been planted.

Soil pH. Wilde, *et al.* (1949) report a suitable range from 4.5 to 6.0 pH as a "planting site requirement" for balsam fir. A significant difference of soil pH was found between sites of heavy and light or no infestation. Two sites, typified by extreme infestations, both had a soil pH above 5.0 (Table 1). Non- or lightly-infested sites were characterized by having a soil pH of between 3.4 and 4.5. There is little doubt that the water table, content of silt and clay particles, and the content of organic matter also must be included in a soil consideration. These were not investigated.

TABLE 1. The soil pH and arbitrary balsam gall midge infestation levels of 10 plots in Oneida County, Wisconsin.

Plot	Location	Infestation level	x̄ soil pH
1	36N 11E 16	Extreme	5.16
2	37N 9E 24	Extreme	5.12
3[1]	37N 10E 30	Moderate	4.56
4	37N 11E 28	Less than 1 percent of twigs	4.53
5	36N 11E 9	None detected	4.39
6	36N 10E 27	None detected	4.33
7	36N 11E 4	Less than 1 percent of twigs	4.24
8	36N 11E 11	Less than 1 percent of twigs	4.01
9	36N 11E 22	Less than 1 percent of twigs	3.74
10	36N 11E 14	Less than 1 percent of twigs	3.44

[1]Average of two samples only; the others five.

Biological Control of the Balsam Gall Midge

Two hymenopterous ectoparasites (Eulophidae), *Tetrastichus whitmani* (Grlt.) and *Tetrastichus marcovitchi* (Cwfd.), have been reared from midge larvae collected in Langlade, Marinette, Oneida, and Sawyer Counties in Wisconsin.[3]

Adults of both species were observed ovipositing on September 7, 1957, in Oneida County, Wisconsin. Adults were moving among the needles on twigs harboring galled needles. Movement was not methodical, but the wasps instinctively selected gall infested needles. After examining the surface of the gall, the oviposition site was selected. The female inserted her ovipositor through the plant tissue, and with legs in a braced position, deposited the egg in the gall chamber. The deposition site varies, occurring on the dorsal and ventral surfaces of the leaf and at any point on those surfaces.

Among 200 galls dissected, seldom was there more than one parasitic larva per gall. Parasitized midge larvae were evidently incapable of migrating; only parasitized and diseased larvae were present after October. Adult parasites did not emerge earlier than December, thus diapause is probably inherent. Transformation to the adult occurs in the spring of the following year, with an alternate host probably carrying it through a first generation. Galls which contained parasitized larvae are easily recognized, as both species cut an escape hole through the gall wall.

In another outbreak in Oneida County, a polyethylene conical trap 5 ft. in diameter was erected beneath a 20-year-old balsam tree. Though midge larval migration had not begun, only 203 midge larvae were obtained from this tree which harbored well over 50,000 larvae. Inspection of the galls revealed parasitism in excess of 80 percent.

Other biological agents. The balsam needle rust, *Milesia marginalis* Faull. and Wats.,[4] indirectly effects control of the midge larvae. This rust is characterized by having white aecia projecting about $\frac{1}{16}$ in. from the under surfaces of the needles, and arising from the stomatal openings. The hyphae grow throughout the needle and eventually fill the gall chamber with a white hyphal mass. Galls on all rust infected needles contained dead larvae.

Chemical Control of the Balsam Gall Midge

Control of the midge presents several important problems. Chemicals directed at the adults and newly hatching eggs involve critical timing, because the imagos are probably very short lived and the field emergence period short. At the time of

[3]The authors are indebted to B. D. Burks, U. S. National Museum, Washington, D. C., for the identification of these species.

[4]Identification by H. C. Green, Dept. of Botany, University of Wisconsin.

year when adults fly, the developing needles would be abnormally susceptible to toxicity. Once enveloped by the gall, the larvae are afforded excellent protection against contact sprays.

In preliminary studies employing systemic insecticides, foliar spray and implantation methods were tested. Systemic preparations, demeton, and Am. Cyanamid CL 12880 (0,0-dimethyl S-methylcarbahoylmethyl phosphorodithioate), gave 100 percent control at 1 gram actual insecticide per tree (Giese *et al.*, 1958). Dimefox caused severe phytotoxicity and R6199 and Thimet required high dosages and too great lengths of time for mortality. No control was obtained following applications of DDT and dieldrin to the foliage. Methods for application of systemic insecticides over large areas have not been developed.

Proposed Survey Procedure

Control of the midge through insecticides or by management has not been reduced to practical levels. Consequently, recommendations for alleviation of losses are directed toward the prevention of badly infested trees reaching the market rather than toward the prevention of infestation. To be successful, such an approach requires a survey technique that will permit rapid assessment of the infestation, and an accurate evaluation of these data in terms of subsequent needle drop and tree marketability. Information developed concerning the distribution of galled needles on the tree, the intensity of needle galling in endemics and epidemics, and the interrrelationships of single and multiple needle infestations has been employed in formulating the survey techniques.[5] Infestation levels and economic considerations relative to the marketability of trees are based upon information supplied by Christmas tree retailers.

Population surveys should be conducted between July 1 and November 1 only in stands between 6 and 20 ft. in height. Open growing stands in low land habitat, plantations, and coppice-managed forests should be examined. Five plots of 4 trees each, distributed at random within each 40-acre area, constitute a sample. One plot per 5 acres will be used for smaller areas. At each plot, the nearest tree in each quadrant should be sampled selecting 4 branches at breast height in the cardinal directions. On each branch 10 current apical twigs should be examined and the number of twigs harboring galled needles recorded. The previous infestation on 10 twigs per tree on 2- and 3-year-old internodes will be estimated also. In old infestations a small portion of the galled needles remain intact indefinitely and other galled needles will be evidenced by abscission scars. Numerous scars reveal a previous heavy infestation.

Damage levels for current foliage are as follows:

1 to 5 percent of twigs infested	LIGHT
6 to 40 percent of twigs infested	MODERATE
41 to 100 percent of twigs infested	HEAVY

Damage levels for foliage on 2 and 3 year internodes are as follows:

90 to 100 percent of needles remaining on old wood. Occasional gall needle present.	LIGHT
50 to 90 percent of needles remaining on old wood. Galled needles present on many twigs.	MODERATE
Less than 50 percent of needles remaining. Galled needles on most twigs.	HEAVY

[5]This survey procedure was submitted to the Lake States Forest Insect Survey Committee in February, 1958, for field testing prior to formal approval.

The data for each stand regarding percent of current needles galled and the infestation level for needles on the 2-and 3-year-old internodes are averaged. The level of the infestation is then derived.

Recommendations for marketing trees are as follows:

1. Trees moderately to heavily infested during the current year will not be marketed.
2. Trees lightly infested during the current year may be marketed if needle galling was no more severe than moderate in the past two years.

Summary

The distribution of the balsam gall midge, *Dasyneura balsamicola* (Lint.), closely parallels the range of its hosts (balsam fir and Fraser fir) throughout North America.

Three larval, the pupal and adult stages are described. Larvae feed throughout the summer and migrate to the ground to overwinter. Pupation and transformation to the adult stage occur in spring. The gall is a monothalamous enlargement, with an *exit orifice*.

In the epidemic situation, typified by having more than one percent of the current foliage being galled, (1) 95 percent of the twigs may be infested, (2) as many as 143 needles per twig may be infested, (3) a terminal preference exists both vertically and radially, and (4) an *infestation locus* comprised of several trees, reveals extreme infestation. Several gall formations are described. These are designated as the typical single, double and triple gall formations and assume the prominent position in terms of frequency. The second major group is composed of the A and B subtypical single, double and triple gall formations. Numerous other variations are formed, with as many as five galls formed per single needle.

Positive significant relationships are demonstrated between (a) the number of galled needles per twig and the number of double and triple galled needles on the same twig, (b) the number of double and triple galled needles, and (c) the number of triple and quadruple galled needles on the same twig.

At the end of the growing season an abscission layer forms, and infested needles drop 3 years prematurely.

Stand conditions associated with epidemics are: (1) soils with a pH above 5.0, and (2) trees (a) of poor general vigor, (b) open growing (c) up to 25 ft. in height, (d) showing an "abnormal" pattern of growth, and (e) having become susceptible due to coppice management or planting.

Midge larvae are attacked by two eulophid ectoparasites, *Tetrastichus whitmani* (Grlt.) and *T. marcovitchi* (Cwfd.) and a balsam needle rust, *Milesia marginalis* Faull. and Wats.

A survey procedure is described including damage levels and recommendations for marketing.

Chemical control of the larvae, which has not been reduced to practical levels, is discussed.

Literature Cited

ALDRICH, J. M. 1905. A Catalogue of North American Diptera. Smith. Inst. Wash., D. C.

BALCH, R. E. 1939. N. S. Dept. Lands-For. R.f. 1938:36.

———. 1940. N. S. Dept. Lands-For. R.f. 1939:35.

BARNES, H. F. 1951. Gall Midges of Economic Importance. Vol. V. Gall midges of Trees. Crosby Lockwood & Son Ltd. London. pp. 72-3.

CHAMBERLIN, W. J. 1946. Entomological Nomenclature and Literature. OSC Coop. Assn. Corvallis, Oreg.

COSENS, A. 1912. A contribution to the morphology and biology of insect galls. Canad. Inst. Trans. 9:297-338.

CRAIGHEAD, F. C. 1950. Insect Enemies of Eastern Forests. U. S. Dept. Agric.

Misc. Pub. 657:509-10.

CURRAN, C. H. 1934. The Families and Genera of North American Diptera. Ballou Press. N. Y.

DAVIAULT, L. 1947. Can. Dept. Agr. Div. Ent. For. Ins. Surv. Can. R.f. 1946:24.

———— and A. R. GOBEIL. 1942. Forest insects of the season 1941 in Quebec. Can. Insect Pest Rev. 20:36-9.

FELT, E. P. 1906. Insects Affecting Park and Woodland Trees. N. Y. St. Mus. Mem. 8(2):685.

————. 1918. Key to American Insect Galls. N. Y. St. Mus. Bull. No. 200:19.

————. 1925. Key to the Gall Midges. N. Y. State Museum Bull. 257.

————. 1940. Plant Galls and Gall Makers. Comstock Pub. Ithaca N. Y.

FOOTE, R. H. 1956. Gall midges associated with cones of western forest trees (Diptera: Itonididae). Wash. Acad. Sci. 46:48-57.

FORBES, R. S. 1957. Maritime provinces. Can. Dept. Agr. Div. Ent. For. Ins. Dis. Surv. R.f. 1956:14.

GIESE, R. L., D. M. BENJAMIN, and J. E. CASIDA. 1958. Results of trunk implantation of systemic insecticides in conifers. J. Econ. Ent. 51(3):400-401.

JARVIS, T. D. 1908. Additional insect galls of Ontario. 37th Rep. Ent. Soc. Ont. 1907:85-94.

LAMBERT, R. 1941. Les Insectes Forestier du Quebec En 1940. Cont. No. 10:23.

————. 1942. Les Insectes Forestier du Quebec En 1941. Cont. No. 15:24.

————. 1949. Can. Dept. Agr. Div. Ent. For. Ins. Surv. R.f. 1948:117.

LEONARD, M. D. 1928. A List of Insects of New York. Cornell Univ. Ag. Exp. Sta. Mem. 101.

LINTNER, J. A. 1888. Fourth Report of the Injurious and Other Insects of the State of New York. (Reprinted from the 41st rept. N. Y. St. Mus.) pp. 60-3.

————. 1890. Report of the State Entomologist. 44th Rept. N. Y. St. Mus. pp. 307-8.

MÖHN, E. 1955. Beitrage zur Systematik der Larven der Itonididae (= Cecidomyiidae) Diptera I. Teil: Porricondylinae und Itonidinae Mitteleuropas. Zoologica. 38 Heft 105. Lieferungen 1 & 2.

PLUMB, G. H. 1953. The formation and development of the Norway spruce gall caused by *Adelges abietis* L. Conn. Ag. Exp. Sta. Bull. 566.

REEKS, W. A., C. C. SMITH and R. S. FORBES. 1947a. Can. Dept. Agr. Div. Ent. For. Ins. Surv. R.f. 1946:14.

————, R. S. FORBES and F. G. CUMING. 1947b. Maritime Provinces. Can. Insect Pest Rev. R.f. Can. Dept. Agr. For. Biol. Div. Sci. Serv. 24:25-9.

WEIGEL, C. A. and R. L. SANDFORD. 1920. The chrysanthemum midge. U. S. Dept. Agric. Bull. 833.

WILDE, S. A., F. G. WILSON and D. P. WHITE. 1949. Soils of Wisconsin in relation to silviculture. Wisc. Cons. Dept. Pub. 525.

WILFORD, B. H. 1929. Insect Pest Survey Bull. U. S. Dept. Agric. 19:(8) 512.

WILLISTON, S. W. 1908. Manual of North American Diptera. 3rd Ed. Hathaway. New Haven.

Suggestions to Contributors

An article submitted for publication should be the best the writer is capable of producing, with all statements, tables, quotations, names and formulas verified before submission. The careful author will have his typescript reviewed by colleagues.

Articles should be typewritten, with 1½-inch margins, on one side only of white bond paper, size 8½ by 11 inches, or 8 by 10½ inches. Carbon copies are not acceptable. All copy should be double-spaced. Pages should be numbered consecutively, preferably in the upper right-hand corner. Only words intended to be set in italics should be underlined.

Material such as equations that exceed 42 spaces on the typewriter cannot be carried in a single line. Such material should either be set so that it can be broken into two or more lines, or should be presented in tabular form.

Tables

Each table should be typewritten on a separate sheet, given a title at the top, and should be numbered consecutively. Even if only one table is submitted, it should be designated Table 1.

Footnotes used in tables should be designated by numerals, never by asterisks or other typographical symbols. These footnotes should be typewritten as part of the table.

Illustrations and Figures

An illustration — whether a photograph, a line drawing, a map, or a graph — is designated as a figure. A caption should be submitted for each illustration, but the caption should be typewritten on a separate sheet.

All figures should be numbered consecutively. If only one is submitted it should be designated Figure 1. On the margin or back of each illustration should be written lightly in soft pencil the number of the figure, the name of the author, and an abbreviated title.

Photographs should be sharp and clear, printed on glossy paper. They should not be smaller than 4 by 5 inches. Sizes 5 by 7 inches and 8 by 10 inches are preferable. Photographs should never be rolled or bent. Care should be taken in fastening photographs with paper clips which often make indentations that show up in reproduction.

Drawings, maps, charts, and graphs are reproduced as line engravings. They should be at least twice as large as they are to appear when reproduced, and should be drawn in india ink on heavy white paper, or tracing cloth.

Footnotes

To indicate a footnote, place a superior figure after the word that refers to the note. Consecutive numerals should be used, never asterisks. Separate the footnote from the text by running a line about one inch inward from the left margin of the type.

Use footnotes to give credit to unpublished material and communications. If only a few references to literature are made (less than one per 1000 words), complete literature citations may be given in footnotes rather than in a separate listing.

Literature Cited

In FOREST SCIENCE practice, references to literature citations are designated by the author's name and year of publication inserted in parentheses at the appropriate place in the text. If there are more than two authors, list only the senior author's name in the text with the abbreviation *et al.* Example: (Smith *et al.*, 1954). Only published references should be given in *Literature Cited*. Periodical abbreviations should follow *Guide to the use of Forestry Abstracts*, Commonwealth Forestry Bureau, Oxford, England, 1950.

Nomenclature and Terminology

When a species is first mentioned in a paper its common name may be immediately followed by its italicized scientific name in parentheses, but this latter name need not be repeated.

As the authority for the exact spelling of tree names, FOREST SCIENCE follows *Check List of Native and Naturalized Trees of the United States (including Alaska)*, Agriculture Handbook 41, Forest Service, U. S. Dept. Agric., Washington, D. C., 1953.

For the spelling of other plant names, contributors should follow *Standardized Plant Names*, 2d Edition, J. Horace McFarland Company, Harrisburg, Pa. 1942.

Technical usage in forestry and allied fields follows *Forestry Terminology*, 3rd Edition, Society of American Foresters, Washington 6, D. C. 1958.

There are many style manuals available for the guidance of writers. One of the best is the *Style Manual* of the U. S. Government Printing Office, Washington 25, D. C. 1953.

Webster's *New International Dictionary* is the accepted authority for general spelling.